

DOCKET NO.: 206269US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :
SHIGERU KAWAHARA ET AL : EXAMINER: ZUCKER
SERIAL NO. : 09/830,159 :
FILED: JUNE 27, 2001 : GROUP ART UNIT: 1621
FOR: CRYSTALLIZATION :
PROCESSES FOR THE
FORMATION OF STABLE
CRYSTALS OF ASPARTAME
DERIVATIVE

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RESPONSE AND REQUEST FOR RECONSIDERATION

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

In response to the Official Action dated September 26, 2002, Applicants respectfully request reconsideration of the above-identified application in view of the following remarks.

REMARKS

At the outset, Applicants wish to thank Examiner Zucker for indicating that the objection to the specification, as set forth in paragraph no. 1 of the previous Official Action; the rejection under 35 U.S.C. § 112, second paragraph, as set forth in paragraph nos. 2-4 of the previous Official Action; the rejection under 35 U.S.C. § 102/103, as set forth in

paragraph no. 5 in the previous Official Action; and the rejection under 35 U.S.C. § 103, as set forth in paragraph no. 6 in the previous Official Action, have all been withdrawn.

Present Claims 5- 11 relate to methods for crystallizing N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester, comprising:

crystallizing N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester from a solution comprising N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester and a solvent, to obtain crystals of N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester which exhibit at least the following diffraction peaks as measured by x-ray diffraction, 2 θ CuK α :

a peak at 6.0°;

a peak at 24.8°;

a peak at 8.2°; and

a peak at 16.5°,

wherein said solvent is selected from the group consisting of water and mixtures of water and a lower alcohol,

wherein said crystallization is carried out such that the temperature of said solution is maintained above 30 °C until onset of nucleation of said N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester.

Present Claims 12-15 relate to methods for crystallizing N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester, comprising:

crystallizing N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester from a solution comprising N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester and a solvent, to obtain crystals of N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-

phenylalanine methyl ester which exhibit at least the following diffraction peaks as measured by x-ray diffraction, 2 θ CuK α :

- a peak at 6.0°;
- a peak at 24.8°;
- a peak at 8.2°; and
- a peak at 16.5°;

wherein said solvent is selected from the group consisting of water and mixtures of water and a lower alcohol,

wherein said crystallization is carried out in the presence of seed crystals of said N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester, and

wherein said seed crystals of said N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester exhibit at least the following diffraction peaks as measured by x-ray diffraction, 2 θ CuK α :

- a peak at 6.0°;
- a peak at 24.8°;
- a peak at 8.2°; and
- a peak at 16.5°.

The inventors have surprisingly found that the presently claimed methods yield an especially stable form of N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester (hereinafter referred to as "neotame"), which is referred to as the A-type crystal form. At the outset, it must be emphasized that the present inventors were the first to find that A-type crystal exhibits excellent stability (see, *e.g.*, page 4, Table 1 and the surrounding description thereof, of the instant specification) as compared to the other polymorphic forms

of neotame such as the B-type, G-type, and the like. The excellent stability of the A-type crystals is, in turn, very useful from the commercial point of view (see, *e.g.*, paragraph bridging pages 1 and 2, of the instant specification).

The cited references contain no disclosure or suggestion of the presently claimed methods or the advantages afforded thereby. Accordingly, these references cannot affect the patentability of the present claims.

The rejection of Claims 5-9 and 11 under 35 U.S.C. § 102(b) or, in the alternative, under 35 U.S.C. § 103(a) in view of U.S. Patent No. 5,510, 508 (Claude et al) and the rejection of Claims 12-15 under 35 U.S.C. § 103(a) in view of Claude et al and further in view of WO 93/02101 (Tosoh) are respectfully traversed.

As shown in Examples 1-5, Claude et al discloses the preparation of neotame by a process in which aspartame is reductively reacted with 3,3-dimethylbutyraldehyde in the presence of a platinum or palladium catalyst in a solvent which is a mixture of a 0.1 M aqueous solution of acetic acid and methanol. After the reaction is complete, the catalyst is removed by filtration, and the pH of the filtrate is adjusted to 5 by the addition of a few drops of a 1 N solution of sodium hydroxide. The methanol is "then removed under vacuum, the temperature being kept below 40°C," and a "white solid rapidly precipitates."

Thus, the precipitation described in Claude et al does not involve carrying out the crystallization "such that the temperature of said solution is maintained above 30 °C until onset of nucleation of said N-[N-(3,3-dimethylbutyl)-L- α -aspartyl]-L-phenylalanine methyl ester." Instead, Claude et al merely discloses that the "temperature being kept below 40°C." Thus, Claude et al only states an upper temperature limit which was not exceeded and says

nothing about any lower temperature limit. In fact, Claude et al never states any actual temperature, only that it is below 40°C. Just how much below 40°C is unstated.

Perhaps even more importantly, Claude et al does not even disclose what temperature is “kept below 40°C.” In this regard, it is evident that Examples 1-5 of Claude et al were carried out on a bench-top scale. Temperature control during the removal of methanol by evaporation under vacuum in such situations is typically achieved by placing the vessel in a water bath and monitoring-controlling the temperature of the water bath. Thus, one of skill in the art would understand that the Examples of Claude et al were carried out by maintaining the temperature of a water bath, not the filtrate itself, below 40°C. In fact, on page 7 of the Official Action, it is stated that the “Examiner agrees with Applicants that this would be a natural assumption....”

Furthermore, as explained in detail in the previously filed response, the method of Claude et al does not yield A-type crystals. For all of these reasons, the methods of the present claims are quite distinct from that of Claude et al. Accordingly, this reference cannot anticipate the present claims.

As for Claims 12-15, Applicants note that these claims recite the use of a specific type of seed crystals (A-type seed crystals). In sharp contrast, Claude et al not only is silent in regard to the use of any type of seed crystals, but also contains no disclosure of A-type crystals or method for preparing A-type crystals.

In support of these assertions, applicants again cite the experiments carried out by Mr. Kawahara as described in the previously filed response and reported in the duly executed declaration under 37 C.F.R. § 1.132 (“the first Kawahara Declaration”). As reported in the first Kawahara Declaration, Mr. Kawahara has carried out a substantial repetition of Example

1 of Claude et al and found that the method resulted in too low a yield of neotame, *i.e.*, a 50-60% yield. Mr. Kawahara then carried out another substantial repetition of Examples 1 and 2 of Claude et al and found that the “white solid” rapidly precipitated in Examples 1 and 2 of Claude et al are also B-type crystals.

On the other hand, Example 1 of Claude et al indeed discloses that “The methanol is then removed by evaporation under vacuum, the temperature being kept below 40°C. A white solid rapidly precipitates.” However, as explained above, it is obvious to those who are skilled in the art that the temperature which kept below 40°C is that of the water in the water bath, and not that of the content or mass in the vessel.

In fact, during his substantial repetitions of Examples 1 and 2 of Claude et al, Mr. Kawahara checked the temperature of the mass in the vessel often and found that the temperature of the mass inside the vessel was, in fact, about 23-28°C, which is below the nucleation temperature of 30°C or greater recited in the present claims. The temperature drop or difference between the bath (40°C) and the mass inside the vessel (about 23°C) is reasonably attributed to the heat of vaporization of the methanol.

For all of these reasons, the present claims are fully patentable over the teachings of Claude et al.

Tosoh indeed discloses the so-called seeding crystallization of Aspartame. However, Tosoh is completely silent in regard to the crystallization of neotame, the use A-type crystals of neotame as seed crystals, or even the existence of A-type crystals of neotame.

On page 9 of the Official Action, it is asserted that “neotame and aspartame would be expected to be very similar.” It is also asserted that it would have been obvious to apply the

seeding method of Tosoh to the crystallization of neotame. However, this logic is incorrect for the following reasons.

First, Applicants agree that Aspartame is somewhat related in structure to neotame. However, neotame is structurally similar to Aspartame only in the sense that neotame contains one Aspartame residue in addition to one 3,3-dimethylbutyl moiety. Therefore, if a chemical reaction involves some reaction site on an Aspartame molecule, neotame might behave like Aspartame in such a reaction. Thus, it might be said that neotame is related in certain chemical properties to Aspartame, only in this sense. Of course, in many chemical properties neotame and Aspartame react quite differently. For example, any reaction which relies on the presence of a $-NH_2$ group will not be possible with neotame.

Applicants also concede that Aspartame and neotame share, to a certain degree, the physiological property of imparting a sweet taste. However, the magnitude of this property is much greater for neotame.

However, Applicants submit that the process of crystallization is not related to the chemical reactivity of certain functional groups present in either Aspartame itself or the aspartame residue contained in neotame. Applicants further submit that crystallization is not a physiological property.

Instead, crystallization is a physical phenomenon, and at most a physico-chemical phenomenon. It is obvious and well known that the physical properties of neotame are quite distinct from those of Aspartame. For example the molecular weight of neotame is about 378 daltons, while that of Aspartame is only about 294 daltons. Moreover, neotame will have a vastly different shape as compared to Aspartame owing to the presence of the additional and relatively voluminous 3, 3-dimethylbutyl group.

When viewed from the perspective of physico-chemical properties, it is seen that the seeding crystallization of Aspartame does not suggest of the presently claimed seeding crystallization of neotame, because Neotame and Aspartame are quite different from each other in physical structure and therefore, in physical properties.

In fact, the different natures of Aspartame and neotame are supported by the disclosure of Tosoh itself. Thus, according to Tosoh, the seeding crystallization of Aspartame is for the purpose of obtaining Aspartame crystals having a larger width to length ratio, no polymorphism being involved therein (see Abstract on the front page of Tosoh). In sharp contrast, the presently claimed seeding crystallization is for the purpose of obtaining the same type, *i.e.*, A- type crystals of neotame as the seed crystals type.

If the assertions in the Official Action were correct, one would naturally expect that the presently claimed seeding crystallization would afford neotame crystals having a larger width to length ratio. This is, however, definitely not the case.

In any event, even if one were to combine the teachings of Tosoh with those of Claude et al, one would still not arrive at the presently claimed seeding method. As noted above, Claude et al contains no disclosure of A-type crystals of neotame or a method of making A-type crystals of neotame. Certainly, there is no disclosure of A-type crystals of neotame in Tosoh. Thus, even if the teaching of these two references were combined, at most one would arrive at a process in which B-type crystals of neotame were used as the seed crystals.

In sharp contrast, present Claims 12-15 explicitly recite the use of seed crystals which "exhibit at least the following diffraction peaks as measured by x-ray diffraction, 2 θ CuK α : a peak at 6.0°; a peak at 24.8°; a peak at 8.2°; and a peak at 16.5°," *i.e.*, A-type crystals.

On page 7 of the Official Action, the position is taken that data in the first Declaration of Kawahara is “not considered persuasive because Applicant presents only selected peak values from the powder x-ray diffraction pattern of Applicants’ A-type crystal.” In response, Applicants cite the data presented in the new duly executed Declaration under 37 C.F.R. § 1.132 filed herewith (“the second Kawahara Declaration”). In the second Kawahara Declaration, the results of the following experiments are reported.

In the Experiment I, Example 2 of Claude et al was repeated with the temperature of the water bath was set at 38-40°C. The product was collected by filtration, and the resulting wet product (5.73 g, moisture 45.92 wt%) was examined by X-ray powder diffractometry.

The complete X-ray diffraction chart of the Neotame crystal obtained by Experiment I is superimposed on the complete chart of the B-type crystals of Neotame (Fig. 2) for the purpose of comparison with the B-type crystals, as shown in Fig. 10. As can be seen from Fig. 10, the crystal of Experiment I is of the B-type. In addition, the complete X-ray diffraction chart of the Neotame crystal obtained by Experiment I was also superimposed on the chart of the A-type crystals of Neotame (Fig. 1), as shown in Fig. 11. As can be seen from Figs. 11 and 12, the crystals of Experiment I contain the B-type crystals but not the A-type crystals.

In Experiment II, Example 2 of Claude et al was repeated with the internal temperature of the solution being maintained not lower than 30°C during the solvent removal. As shown in the second Kawahara Declaration, Experiment II also gave B-type crystals but not A-type crystals (see complete diffraction patterns shown in Figs. 13 and 14).

In summary, as shown in the second Kawahara Declaration, the method disclosed in Claude et al does not inherently afford A-type crystals of Neotame.

For these reasons, the rejections should be withdrawn.

The rejection of Claims 5-15 under 35 U.S.C. § 112, second paragraph, is respectfully traversed. On page 10 of the Official Action, the position is taken that the present claims are indefinite due to the recitation of "selected peak values as characteristic of the x-ray diffraction pattern of a particular crystalline form." However, Applicants submit that the use of selected peak values is accepted by those of skill in the art for identifying a specific crystalline form of a particular compound.

In support of this assertion, Applicants cite U.S. Patent No. 4,579,747 (Sugiyama et al, copy attached hereto as Exhibit A), which issued nearly 17 years ago, in which claims identifying two types of Aspartame crystals (IIA and IIB) by the recitation of just four (4) diffraction peaks were allowed. Similarly, in U.S. Patent No. 4,950,780 (Tanaka et al, copy attached hereto as Exhibit B), issued over twelve (12) years ago, a novel crystalline structure of n-octadecyl-3-(3,5-di-t-butyl-4-hydroxyphenyl)-propionate was claimed by the recitation of only one (1) diffraction peak. Likewise, in European Patent No. 0 279 542 B1 (copy attached hereto as Exhibit C), which issued on August 12, 1991, over eleven (11) years ago, the B-type crystals of aspartic acid were defined in the claims by the identification of only three (3) diffraction peaks.

In addition, Applicants also cite the duly executed Declaration under 37 C.F.R. § 1.132 of Kashiwagi which was executed October 31, 2002 and filed in co-pending U.S. Patent Application Serial No. 09/708,006 ("the Kashiwagi Declaration"), a copy of which is attached hereto as Exhibit D. As explained in the Kashiwagi Declaration, the powder X-ray diffraction method is widely used among those skilled in the art to identify the crystal type of a particular compound.

Thus, the identification of crystal types by recitation of several peculiar or specific diffraction peaks in the powder diffraction X-ray method is well accepted by those who are skilled in the art, including U.S. patent examiners.

Moreover, the specific peaks recited in the present claims as particularly useful for the identification of the claimed crystals. As explained in the second Kawahara Declaration, analysis of the A-type crystals of Neotame obtained by the crystallization method of the present claims by the powder diffraction X-ray method gives the chart shown in Fig. 1. In contrast, analysis of the B-type crystal of Neotame obtained by repeating Example 1 of U.S. Patent No. 5,728,862 (Prakash et al) by the same procedures gives the chart shown in Fig. 2. The diffraction pattern shown in Fig. 1 (A-type crystals) are compared with that of Fig. 2 (B-type crystals) as described below.

First of all, the relatively high peaks of the A-type crystals were assigned Nos. A1-A20, respectively, as indicated in Fig. 3 (A-type crystals), for the sake of convenience of explanation in making the comparison. The diffraction angles and relative intensity of peaks Nos. A1-A20 were collected and are listed in Table 1, below. Similarly, the relatively high peaks of the B-type crystals were assigned Nos. B1-B18, respectively, as shown in Fig. 4 (B-type), and are listed in Table 2 below.

TABLE 1(A-Type Crystals)

No.	<u>Diffraction angle</u>	<u>Relative intensity</u>
	2θ	$I/I_1(\%)$
A1	5.95	100
A2	8.22	28
A3	12.41	7
A4	14.21	28
A5	16.54	59
A6	16.88	24
A7	17.69	32
A8	19.80	17
A9	20.42	76
A10	20.88	22
A11	21.31	14
A12	21.92	14
A13	22.94	40
A14	23.82	23
A15	24.76	17
A16	25.00	16
A17	26.00	41
A18	26.32	32
A19	26.67	29
A20	28.69	25

TABLE 2 (B-Type Crystals)

No.	<u>Diffraction angle</u>	<u>Relative intensity</u>
	2 θ	I/I ₁ (%)
B1	5.18	100
B2	8.42	18
B3	10.24	35
B4	11.95	8
B5	14.23	3
B6	15.69	8
B7	16.36	12
B8	17.45	7
B9	18.25	25
B10	20.51	4
B11	21.10	26
B12	21.39	18
B13	21.88	14
B14	23.94	5
B15	25.30	7
B16	25.67	6
B17	26.85	5
B18	27.28	9

The inventors selected the following four (4) peaks to identify the A-type crystals, from Table 1 and Fig. 3: 6.0° (A1), 8.2° (A2), 16.5° (A5), and 24.8° (A15). It is seen from Table 2 and Fig. 4 that none of these four peaks appear in the chart of the B-type crystals.

The fact that the four peaks selected as being characteristic of the A-type crystals and the peaks characteristic to the B-type crystals will appear as separate peaks on one X-ray powder diffraction chart was demonstrated by the following experiment. B-type crystals and the A-type crystals were each separately prepared and then mixed, and the resulting mixtures were analyzed by the powder diffraction X-ray method. The diffraction pattern of the obtained A-type crystal (moisture 34.0 wt%) and that of the obtained B-type crystal (moisture 58.7 wt%) are shown in Figs. 5 and 6 of the second Kawahara Declaration, respectively.

The mixtures of the A-type crystals and the B-type crystals were prepared and analyzed, as follows;

- | | |
|------------|--|
| Mixture 1: | 0.80 g of the B-type crystals and 0.24 g of the A-type crystals were placed in a mortar and mixed. When analyzed by the powder diffraction X-ray method, the mixture gave the chart shown in Fig. 7, of the second Kawahara Declaration. |
| Mixture 2: | A mixture of 1.0 g of the B-type crystals and 0.1 g of the A-type crystals was prepared in the same manner and gave the diffraction pattern shown in Fig. 8, of the second Kawahara Declaration. |

It is clear from Figs. 7 and 8 of the second Kawahara Declaration that the four peaks A1, A2, A5, and A15 of the A-type crystals selected by the present inventors as being characteristic of the A-type crystals are clearly distinguishable from the peaks characteristic of the B-type crystals. Moreover, it is particularly noteworthy that, in Mixture 2, the four

peaks A1, A2, A5, and A15 are clearly distinct on the chart shown in Fig. 8, notwithstanding the fact that the A-type crystals were contained in an amount of as little as about 14 % based on the B-type crystals.

In view of the foregoing, it can be said concluded that when given crystals of Neotame are analyzed by the powder diffraction X-ray method, if the four peaks A1, A2, A5, and A15 selected by the present inventors as being characteristic of the A-type crystal are not recognized or do not appear in the diffraction pattern, then the crystals are not of the A-type.

As also explained in the second Declaration of Kawahara, there is another technique generally employed among those who are skilled in the art, for the purpose of comparison of two charts obtained by the powder diffraction X-ray method. In this method, one chart is superimposed on the other such that the X axes (2θ , $\text{CuK}\alpha$ line) just overlap each other, whereby the peaks of the one chart and those of the other can be precisely and easily compared. In accordance with this technique, the chart of the A-type crystals of Neotame (Fig. 1) and that of the B-type crystals of Neotame (Fig. 2) are shown on one sheet in such an overlapping position in Fig. 9 of the second Kawahara Declaration. As shown in Fig. 9, the four peaks selected by the present inventors as being characteristic of the A-type crystals are clearly distinguishable from the peaks characteristic of the B-type crystals.

In view of the foregoing, it is seen that the four peaks A1, A2, A5, and A15 are sufficient to identify the A-type crystals of Neotame.

For all of these reasons, the rejection is improper and should be withdrawn.

Applicants submit that the application is now in condition for allowance, and early notification of such action is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

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22850

A handwritten signature in black ink, appearing to read 'Stephen G. Baxter' with a stylized flourish at the end.

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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :
SHIGERU KAWAHARA ET AL : EXAMINER: ZUCKER
SERIAL NO. : 09/830,159 :
FILED: JUNE 27, 2001 : GROUP ART UNIT: 1621
FOR: CRYSTALLIZATION :
PROCESSES FOR THE
FORMATION OF STABLE
CRYSTALS OF ASPARTAME
DERIVATIVE

DECLARATION UNDER 37 C.F.R. § 1.132

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Now comes Shigeru Kawahara, who deposes and states:

1. That I am a named inventor of the above-identified application.
2. That I received my degree in chemistry from Tokyo Institute of Technology in 1998.
3. That I have been employed by Ajinomoto Company, Inc., the assignee of the above-identified application for 5 years as a chemist in the field of process chemistry.
4. That I am the same Shigeru Kawahara who executed a first declaration under 37 C.F.R. § 1.132 in this application on July 19, 2002.
5. Even in the same material, several kinds of crystalline states (crystal forms) can exist. X-ray powder diffraction (XRPD) has long been used to identify and characterize

crystalline powders. In order to discriminate between crystalline powder samples which commonly contain the same material but belong to the different crystal forms, XRPD is the most powerful and convenient method. The XRPD patterns of the same materials belonging to different crystal forms demonstrate diffraction peaks with different 2θ angles.

6. It is a conventional identification method for a specific crystal to find crystal(s) having the same three strongest peaks of the specific crystal on XRPD data bases and, when found, to analyze coincidence of other peaks between them. In general cases, a crystal can be identified when the strongest eight peaks are coincident. In the case of the specific crystals of a particular compound, for instance, A-type crystals, B-type crystals, or other types crystals of Neotame, it is possible to identify any one of them by using one strong and characteristic peak, as the case may be.

7. The identification of crystal types by XRPD (or by several characteristic or specific diffraction peaks obtained therein) is well accepted by those who are skilled in the art, as a means for the identification of crystal types. For example, in U.S. Patent No. 4,579,747, which issued on April 1, 1986, two types of Aspartame crystals (IIA and IIB) were claimed by identification of four (4) diffraction peaks. In U.S. Patent No. 4,950,780, which issued on August 21, 1990, a novel crystalline structure of n-octadecyl-3-(3,5-di-t-butyl-4-hydroxyphenyl)-propionate was claimed by the identification of only one (1) diffraction peak. In European Patent No. 0 279 542 B1, granted on August 12, 1991, β -type crystals of aspartic acid were claimed in terms of only three (3) diffraction peaks.

8. As has been described above, XRPD is widely used among those skilled in the art to identify the crystal type of a compound. Peaks obtained by XRPD reflect the internal structure of the crystal concerned, and the obtained peaks are peculiar or specific to the

crystal. Therefore, for the identification of the crystal type of a given compound, not all the peaks need to be assigned, and it suffices that some typical peaks are consistent with those of an authentic sample.

9. To demonstrate that the peaks at 6.0° , 8.2° , 16.5° , and 24.8° are sufficient to identify A-type crystals of Neotame, the following experiments were carried out by me or under my direct supervision and control.

(A) First, the A-type crystals of Neotame obtained by the crystallization method of the present invention were analyzed by XRPD, and this analysis gave the chart shown in Fig.

1. The B-type crystals of Neotame obtained by repeating Example 1 of U.S. Patent No. 5,728,862 (Prakash et al) were analyzed by XRPD, and this analysis gave the chart shown Fig. 2.

(B) The diffraction pattern of the A-type crystals shown in Fig. 1 was compared with that of the B-type crystals shown in Fig. 2 as follows. First, the relatively strong peaks of the A-type crystals were assigned Nos. A1-A20, respectively, as indicated in Fig. 3 (A-type crystals), for the sake of convenience of explanation in making the comparison. The diffraction angles and relative intensity of peaks Nos. A1-A20 were collected and are listed in Table 1. Similarly, the relatively intense peaks of the B-type crystals were assigned Nos. B1-B18, respectively, as indicated in Fig. 4 (B-type crystals), and are listed in Table 2.

TABLE 1 (A-Type Crystals)

No.	<u>Diffraction angle</u>	<u>Relative intensity</u>
	2θ	$I/I_1(\%)$
A1	5.95	100
A2	8.22	28
A3	12.41	7
A4	14.21	28
A5	16.54	59
A6	16.88	24
A7	17.69	32
A8	19.80	17
A9	20.42	76
A10	20.88	22
A11	21.31	14
A12	21.92	14
A13	22.94	40
A14	23.82	23
A15	24.76	17
A16	25.00	16
A17	26.00	41
A18	26.32	32
A19	26.67	29
A20	28.69	25

TABLE 2 (B-Type Crystals)

No.	<u>Diffraction angle</u>	<u>Relative intensity</u>
	2 θ	I/I ₁ (%)
B1	5.16	100
B2	8.42	18
B3	10.24	35
B4	11.95	8
B5	14.23	3
B6	15.69	8
B7	16.36	12
B8	17.45	7
B9	18.25	25
B10	20.51	4
B11	21.10	26
B12	21.39	18
B13	21.88	14
B14	23.94	5
B15	25.30	7
B16	25.67	6
B17	26.85	5
B18	27.28	9

The following four (4) peaks were selected from Table 1 and Fig. 3 to identify the A-type crystals: 6.0° (A1), 8.2° (A2), 16.5° (A5) and 24.8° (A15) (2 θ , CuK α line). It is clear from Table 2 and Fig. 4 that none of these four peaks appear in the chart of the B-type crystals (Fig 4).

(C) To demonstrate empirically that the four peaks selected as being characteristic of the A-type crystals and the peaks characteristic of the B-type crystal are distinguishable on one chart, the B-type crystals and the A-type crystals were each separately prepared and were mixed, and the resulting mixtures were analyzed by XRPD. The A-type and B-type crystals were prepared by repeating Example 4 and Comparative Example 1, respectively, of the above-identified application. The XRPD chart of the obtained A-type crystals (moisture 34.0 wt%) and that of the obtained B-type crystals (moisture 58.7 wt%) are shown in Figs. 5 and 6, respectively.

Mixtures of the A-type crystal and the B-type crystal were prepared and analyzed, as follows:

Mixture 1: 0.80 g of the B-type crystals and 0.24 g of the A-type crystals were placed in a mortar and mixed. The mixture gave the chart of Fig. 7, when analyzed by XRPD.

Mixture 2: A mixture of 1.0 g of the B-type crystals and 0.1 g of the A-type crystals was prepared in the same manner and gave the chart of Fig. 8, when analyzed by XRPD.

As seen from Figs. 7 and 8, the four peaks A1, A2, A5, and A15 of the A-type crystals selected by the present inventors as being characteristic of the A-type crystal are clearly distinguishable from the peaks characteristic of the B-type crystal, in both Mixtures 1 and 2. It is particularly noteworthy that, in Mixture 2, the four peaks A1, A2, A5, and A15 are distinguishable on the chart of Fig. 8, notwithstanding the fact that the A-type crystals were contained in an amount of as little as about 14 % based on the B-type crystals.

(D) In view of the foregoing, it can be safely concluded that when given crystals of Neotame are analyzed by the powder diffraction X-ray method, and if the four peaks A1, A2, A5, and A15 selected by the present inventors as being characteristic of the A-type crystals are not recognized or do not appear on the chart, then the crystals do not contain the A-type crystals.

(E) Superimposition of two charts is a technique generally employed among those who are skilled in the art, for the purpose of comparison of two charts obtained by XRPD. Specifically, one chart is placed on the other such that the X axes (2θ , $\text{CuK}\alpha$ line) just overlap each other, whereby the peaks of the one chart and those of the other can be precisely and easily compared. In accordance with this technique, the chart of the A-type crystals of Neotame (Fig. 1) and that of the B-type crystals of Neotame (Fig. 2) are shown on one sheet in such an overlapping position; in Fig. 9. As seen from Fig. 9, the four peaks selected by the present inventors as being characteristic of the A-type crystal are clearly distinguishable from those characteristic of the B-type crystal, unless the A-type crystal is really mixed with the B-type crystal.

In view of the foregoing, it could be concluded that the four peaks A1, A2, A5, and A15 selected by the present inventors as being characteristic of the A-type crystals of Neotame are sufficient for the identification of the A-type crystals of Neotame.

10. The following additional experiments were also carried out by me or under my direct supervision and control.

11. Experiment I: Repetition of Example 2 of U.S. Patent No. 5,510,508 (Claude et al), with the temperature of the water bath maintained at 38-40°C during the precipitation of the Neotame. The following were introduced successively, with stirring, into a reactor

equipped with a stirrer capable of ensuring a very good transfer of gaseous hydrogen into the liquid phase: 60 cm³ of a 0.1 M aqueous solution of acetic acid, 1 g of 10 % palladium on activated carbon, 2.55 g of 3,3-dimethylbutyraldehyde, 30 cm³ of methanol, and 5 g of aspartame. After the reactor had been purged with a stream of nitrogen, the mixture was hydrogenated at a relative pressure of 1 bar (0.1 MPa) and at room temperature. The progress of the reaction was monitored by taking a crude sample and assaying the product formed by high performance liquid chromatography (HPLC). The concentration of desired product was determined by comparison with a calibration curve established beforehand. After a hydrogenation time of 2 hours, the formation of 56 % of the expected product was observed.

The reaction was then interrupted by purging the reactor with a stream of nitrogen, and the catalyst was removed by filtration with a fine filter (0.5 μm). The methanol was then removed by evaporation under vacuum, while the temperature of the water bath was maintained at 38-40°C. During the removal of methanol by evaporation under vacuum, the temperature of the mass inside the vessel was checked by a thermometer. After 3-13 minutes, an oily material was generated, and after another 1 minutes (14 minutes from starting evaporation), a white solid rapidly precipitated. At that time, the temperature of the mass inside the vessel was 23°C. The mixture was stirred for five hours at room temperature in order to complete the precipitation. The product was collected by filtration, and the resulting wet product (5.73 g, moisture 45.92 wt%) was examined by XRPD.

The X-ray diffraction chart of the Neotame crystals obtained by Experiment I was superimposed on the chart of the B-type crystals of Neotame (Fig. 2) for the purpose of comparison with the B-type crystals, as shown in Fig. 10. As can be seen from Fig. 10, the crystals of Experiment I are of the B-type, because of the appearance on the chart of peaks B1,

B2, B11, and B12 characteristic of the B-type crystals. However, new peaks X1 ($2\theta = 5.64$) and X2 ($2\theta = 8.33$) were also observed, and therefore, the crystal is probably mixed with some unknown type crystal.

Subsequently, the X-ray diffraction chart of the Neotame crystals obtained by Experiment I was superimposed on the chart of the A-type crystals of Neotame (Fig. 1) for the purpose of comparison with the A-type crystals, as indicated in Fig. 11. As can be seen from Fig. 11, none of the peaks A1, A2, A5, and A15 selected by the present inventors as being characteristic of the A-type crystals are observed on the chart of the crystals of Experiment I.

In conclusion, the crystals of Experiment I are a mixture of B-type crystals and some unknown type crystal and are not A-type crystals.

12. Experiment II: Repetition of Example 2 of Claude et al with the solvent maintained at a temperature of not lower than 30°C during solvent removal. The following were introduced successively, with stirring, into a reactor equipped with a stirrer capable of ensuring a very good transfer of gaseous hydrogen into the liquid phase; 353 cm³ of a 0.1 M aqueous solution of acetic acid, 5.88 g of 10 % palladium on activated carbon, 15.00 g of 3,3-dimethylbutyraldehyde, 177 cm³ of methanol, and 30.64 g of aspartame. After the reactor had been purged with a stream of nitrogen, the mixture was hydrogenated at a relative pressure of 1 bar (0.1 MPa) and at 30°C. The progress of the reaction was monitored by taking a crude sample and assaying the product formed by high performance liquid chromatography (HPLC). The concentration of desired product was determined by comparison with a calibration curve established beforehand. After a hydrogenation time of 19 hours, the formation of 47.7 % of the expected product was observed. The reaction was then interrupted

by purging the reactor with a stream of nitrogen, and the catalyst was removed by filtration with a fine filter ($0.5\mu\text{m}$). The catalyst was washed by methanol (10 cm^3) and water (20 cm^3), and a combined filtrate (600 cm^3) containing 18.0 g of Neotame (yield 47.5 %) was obtained. 50 cm^3 of the resulting filtrate was used for the following concentration-crystallization process.

The above filtrate containing 1.5 g of Neotame was introduced into a four-neck round-bottom flask (200 cm^3) equipped with a stirrer and a thermometer capable of measuring the temperature of the solution inside the flask. The methanol was then removed under reduced pressure (25-100 mm Hg) for 3.5 hours so that the temperature of the solution inside the flask was allowed to drop slowly to 30°C from 42°C . The nucleation did not occur during concentration, although a viscous oil was formed out of the solution.

The flask was detached and held at room temperature for about 20 minutes. The viscous oil began to change to crystals, and the temperature inside the flask was 27.6°C . The mixture was stirred for a while in order to complete the precipitation. The product was collected by filtration, and the resulting wet-product (1.74 g, moisture 31.91 wt%) was examined by XRPD.

Thus, in Experiment II, no crystals were precipitated, notwithstanding the fact that the solvent was removed by evaporation while the internal temperature of the solvent was not lower than 30°C , *i.e.*, the temperature of the solution inside the flask, over a period of 3.5 hours. It was, thus, found that as the result of the prolonged concentration time, an oily substance insoluble in water was precipitated on, and adhered to, the inside wall of the flask, and converted to crystals only when cooled down to room temperature.

According to conventional crystallization, nucleation is caused to occur from a homogeneous solution by causing a supersaturation gradually. In contrast, in Example 2 of Claude et al, an oily substance is first formed and then converted to crystals.

The x-ray diffraction chart of the crystals obtained by Experiment II is shown in Fig. 12. The X-ray diffraction chart of the Neotame crystals obtained by Experiment II (Fig. 12) was then superimposed on the chart of the B-type crystals of Neotame (Fig. 2) for the purpose of comparison with the B-type crystals, as indicated in Fig. 13. As can be seen from Fig. 13, the crystals of Experiment II contain the B-type crystals, as indicated by the appearance of peaks B1, B3, B9, B11, and B12, which are characteristic of the B-type crystals. There were also observed, however, the additional peaks X1 ($2\theta = 5.64$) and X2 ($2\theta = 8.33$), and the relative intensities of these peaks were stronger than that in Experiment I, and therefore, the crystals are probably mixed with some unknown type crystal in a larger amount than the crystals of Experiment I.

The x-ray diffraction chart of the Neotame crystal obtained by Experiment II (Fig. 12) was also superimposed on the chart of the A-type crystals of Neotame (Fig. 1) for the purpose of comparison with the A-type crystals, as indicated in Fig. 14. As can be seen from Fig. 14, none of the four peaks A1, A2, A5, and A15 selected by the present inventors as being characteristic of the A-type crystals are observed on the chart of the crystal of Experiment II.

In conclusion, repetition of Example 2 of Claude et al with maintaining the temperature of the solution at a temperature of not lower than 30°C during concentration gives a mixture of the B-type crystals and some unknown type crystal, and does not afford A-type crystals.

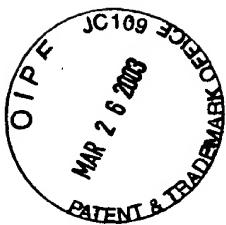
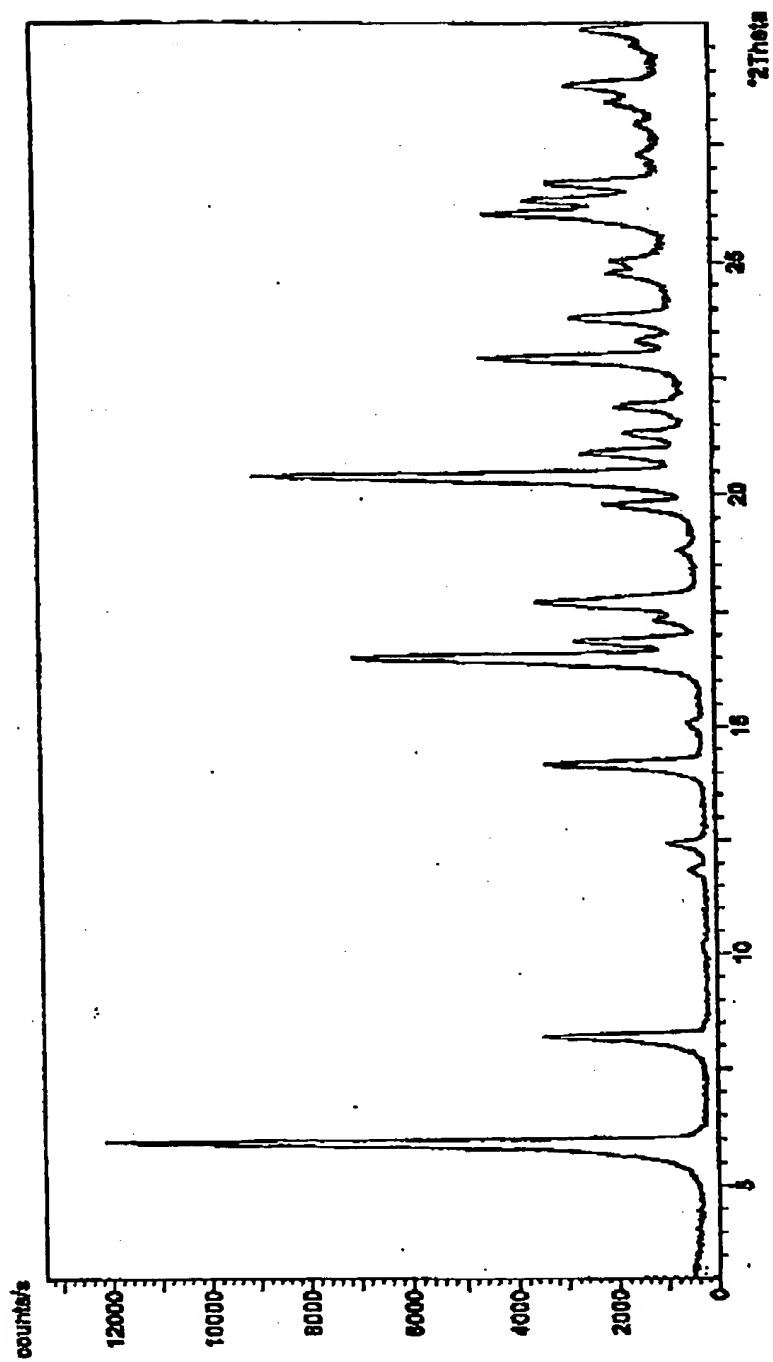


FIGURE 1



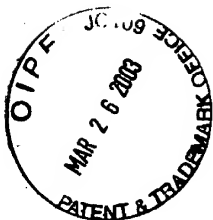


FIGURE 2

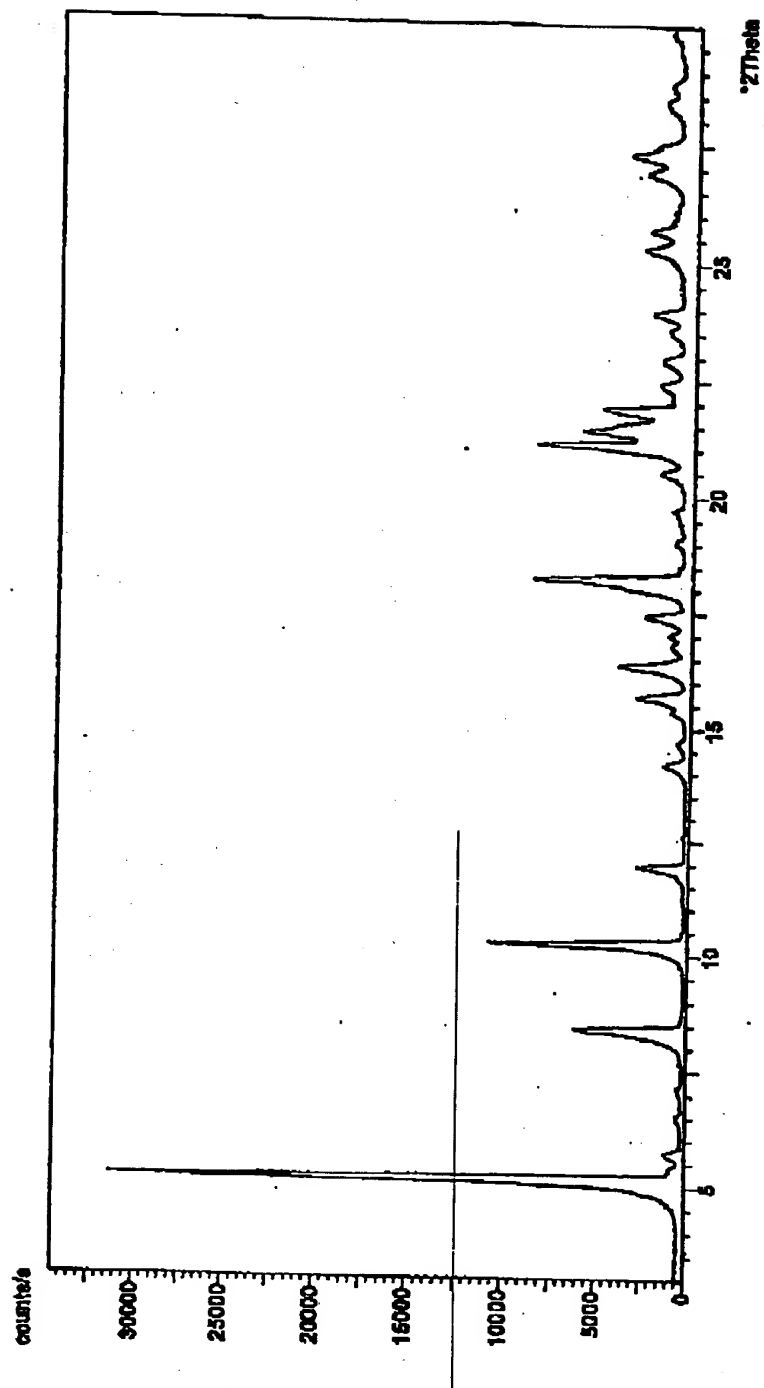
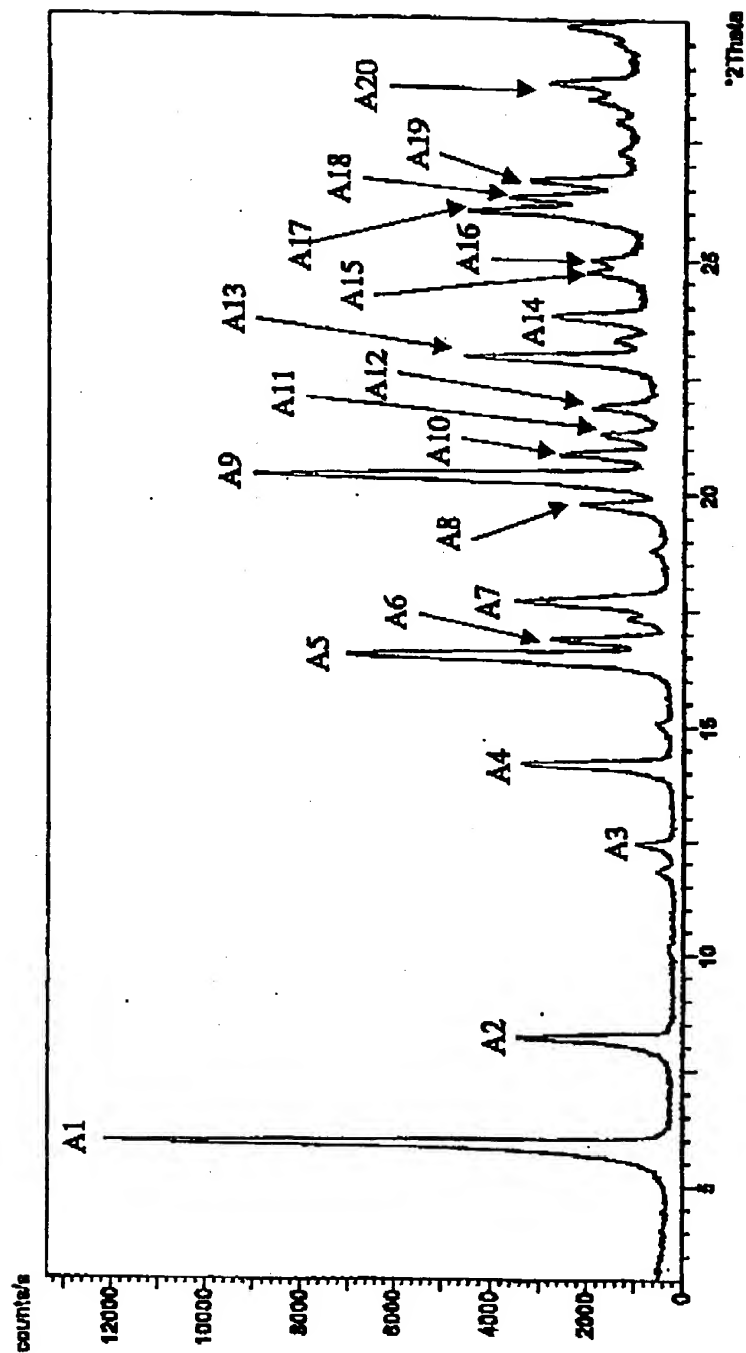


FIGURE 3



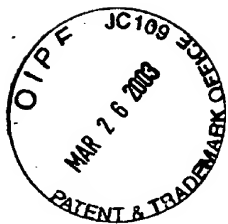
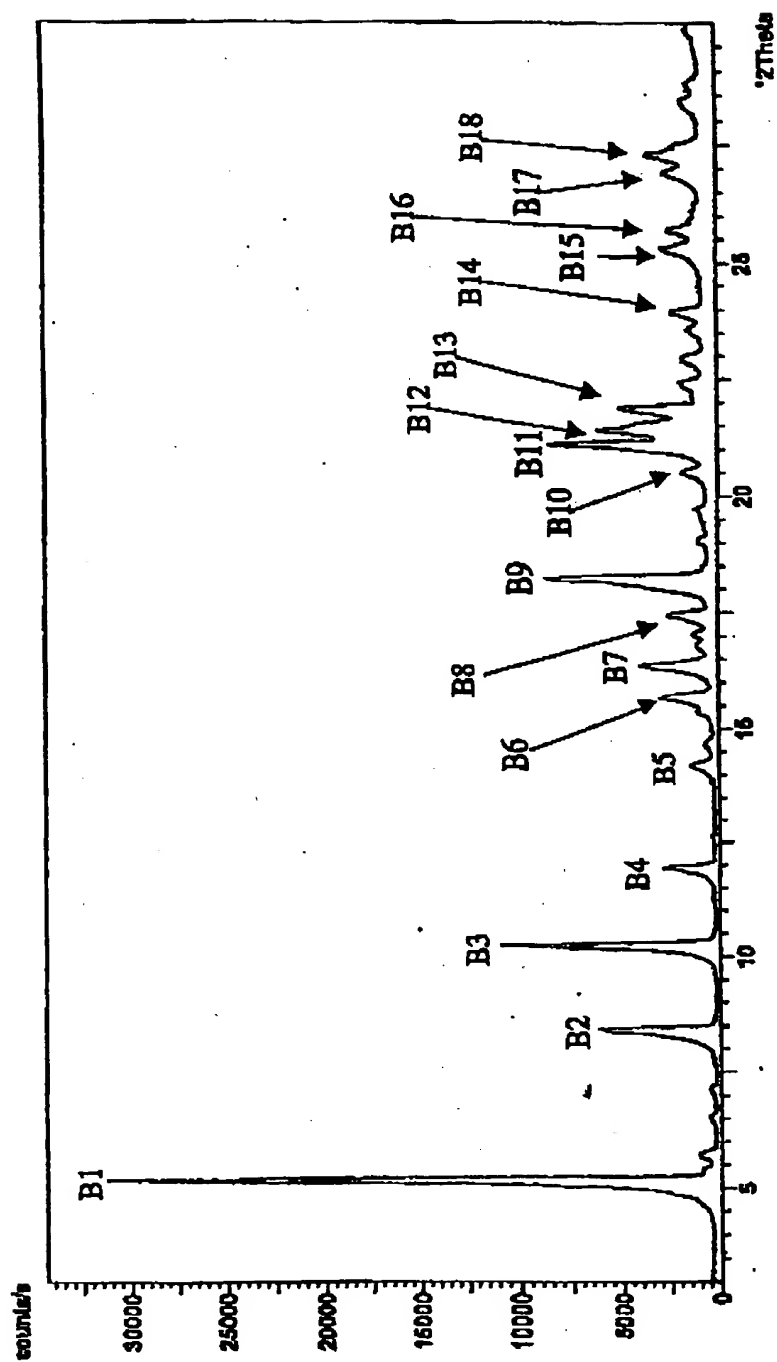


FIGURE 4



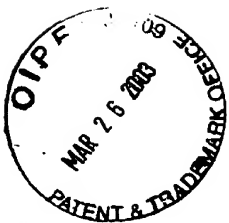
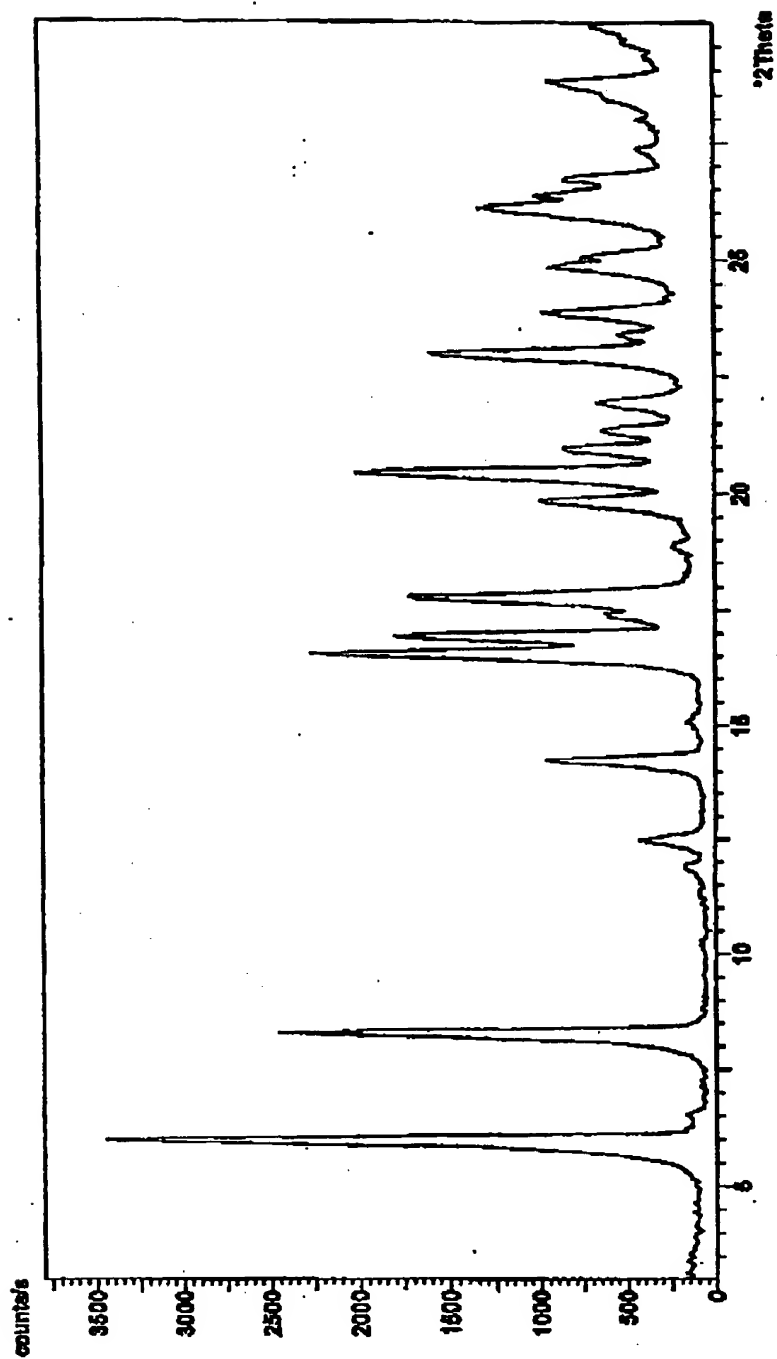


FIGURE 5



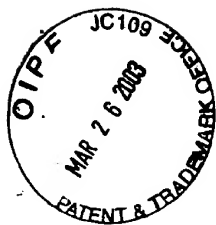


FIGURE 6

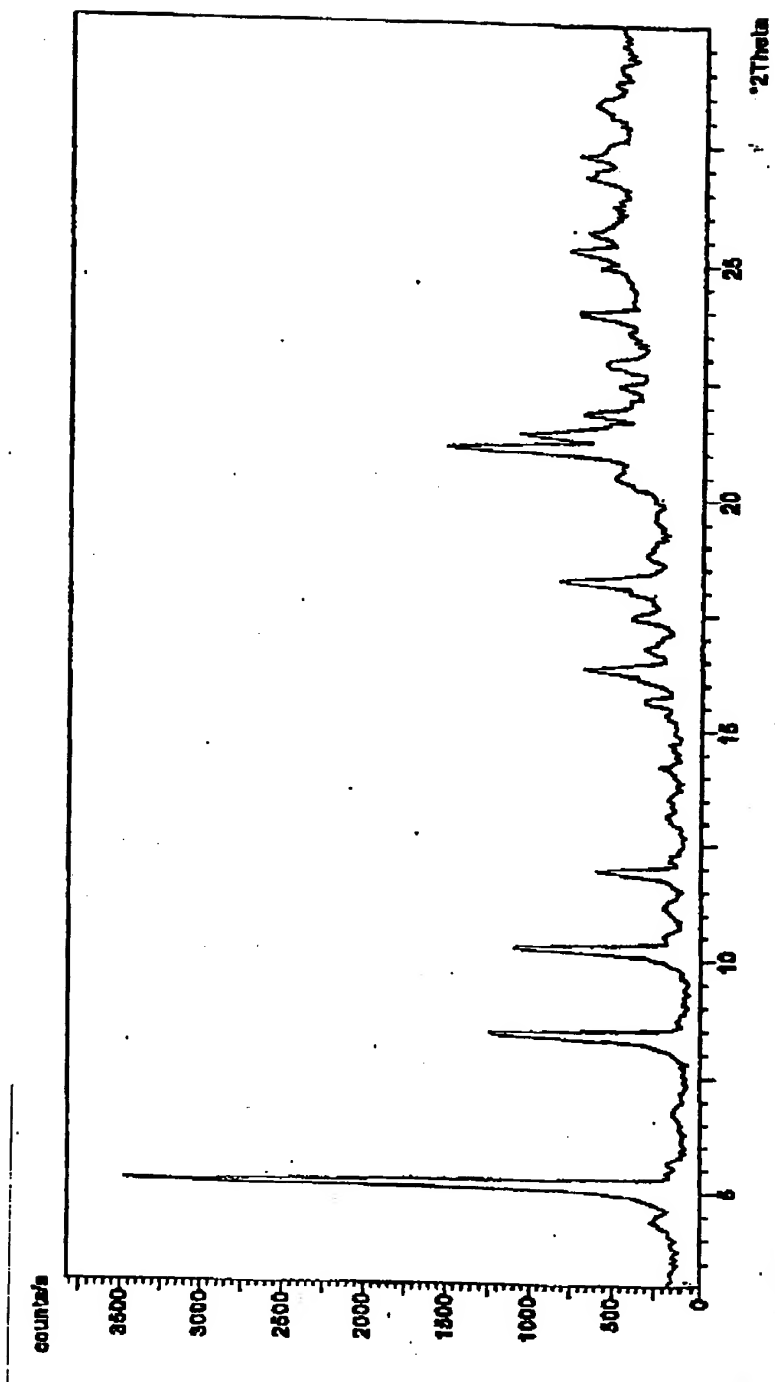
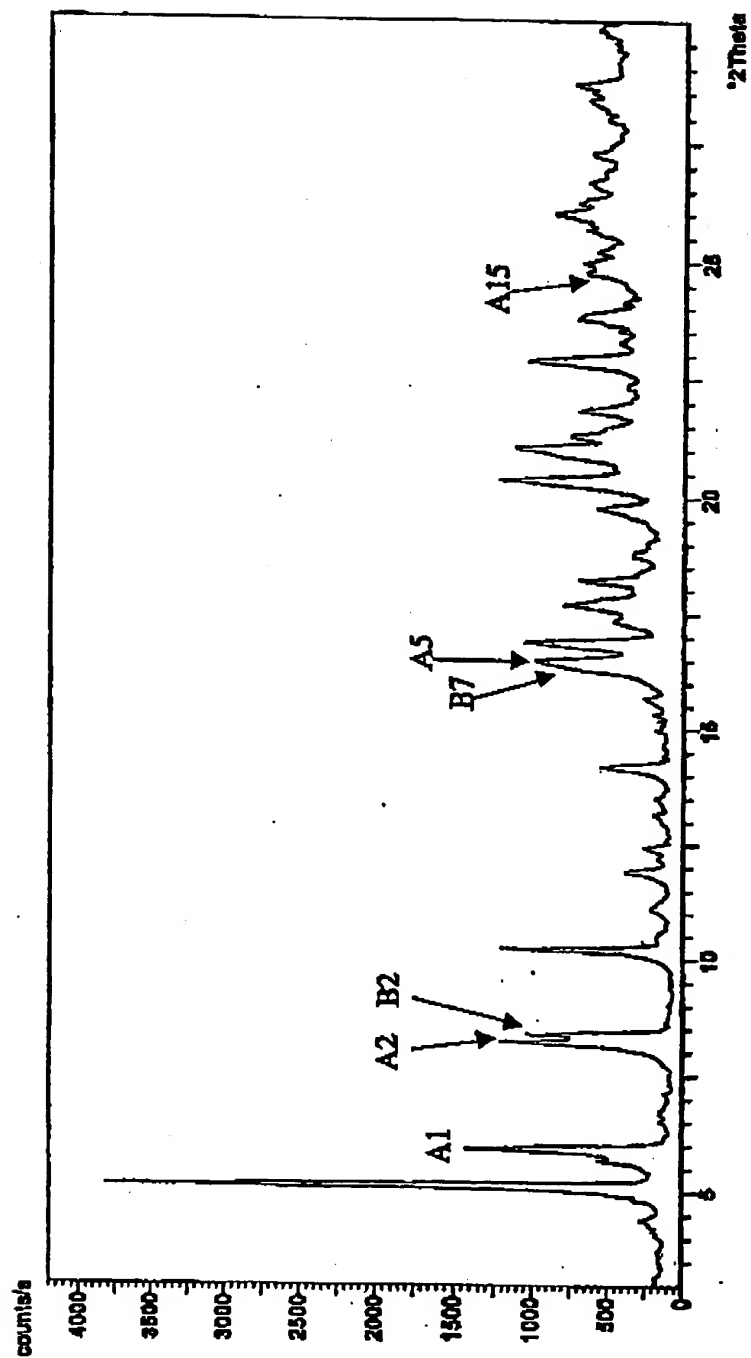




FIGURE 7



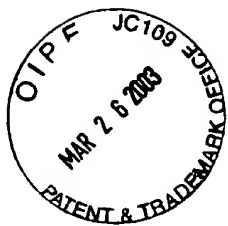


FIGURE 8

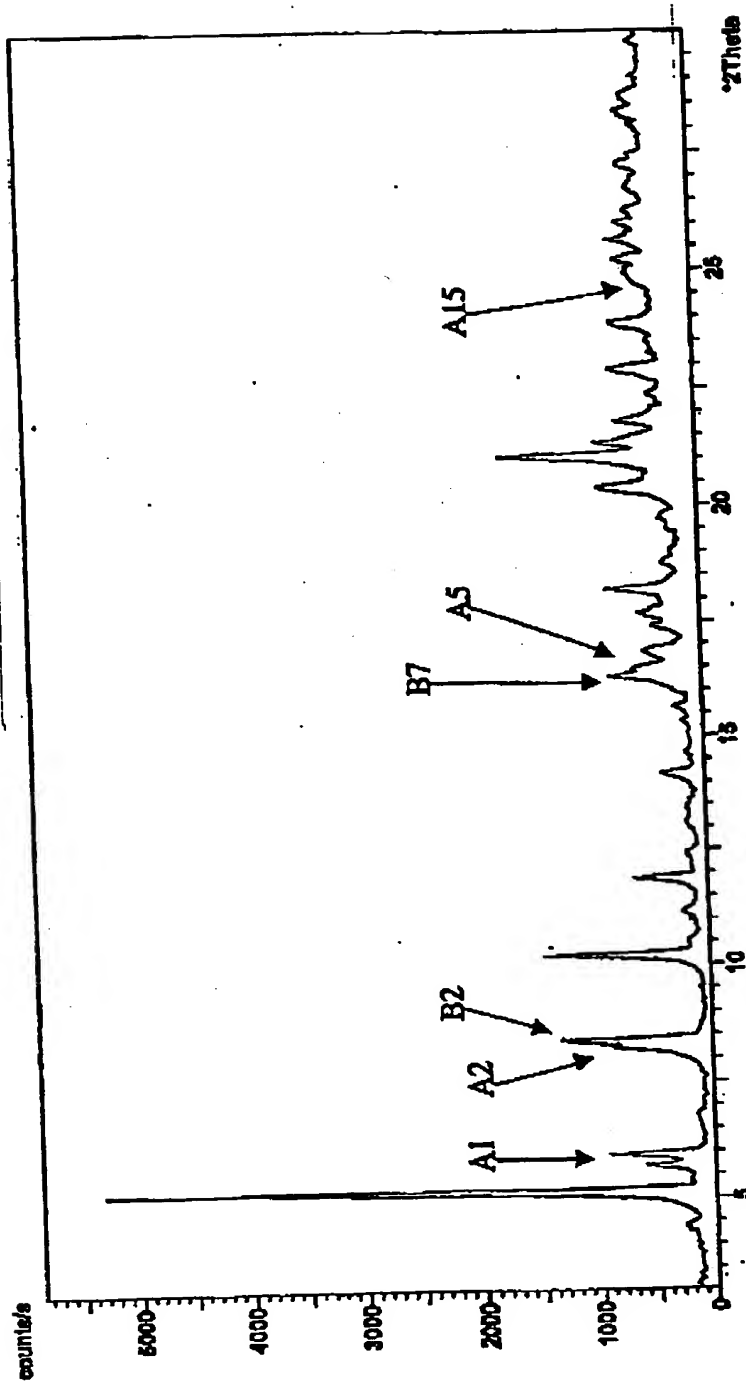




FIGURE 9

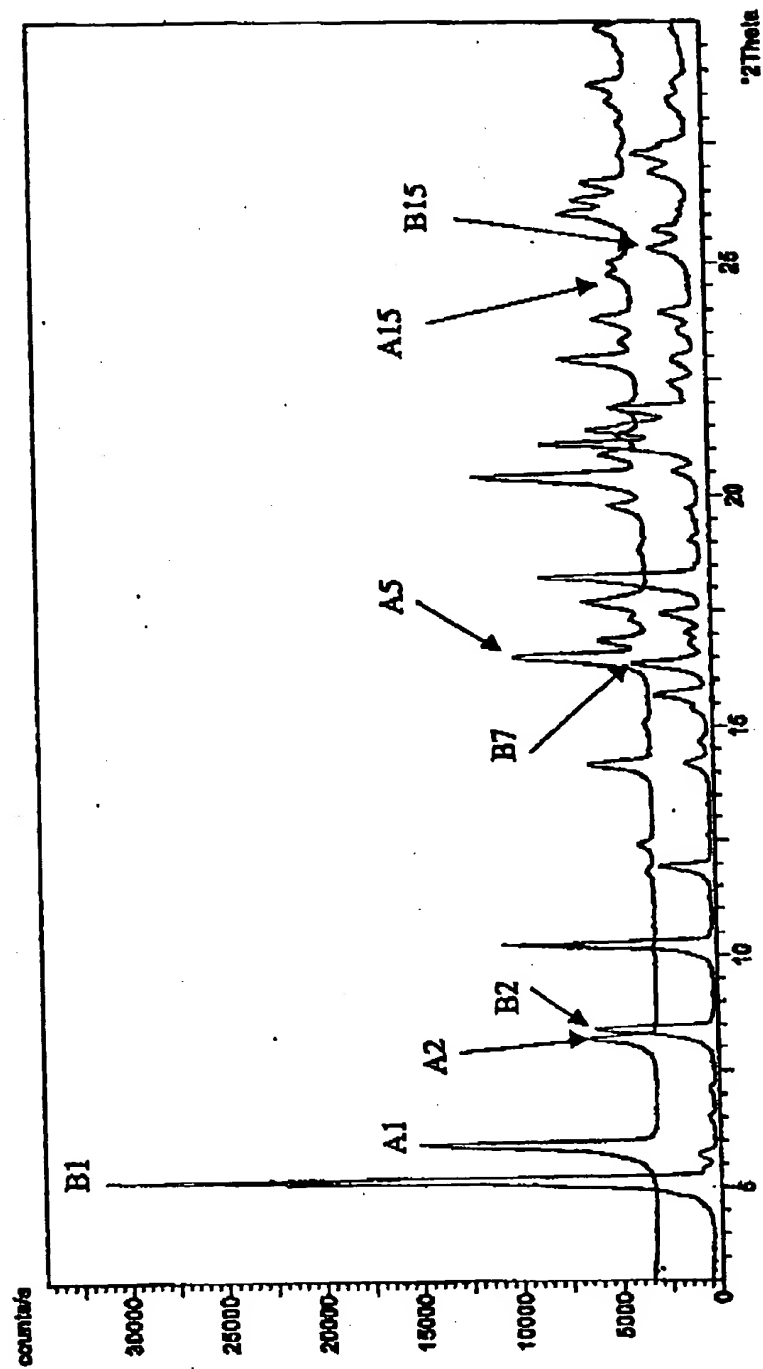


FIGURE 10

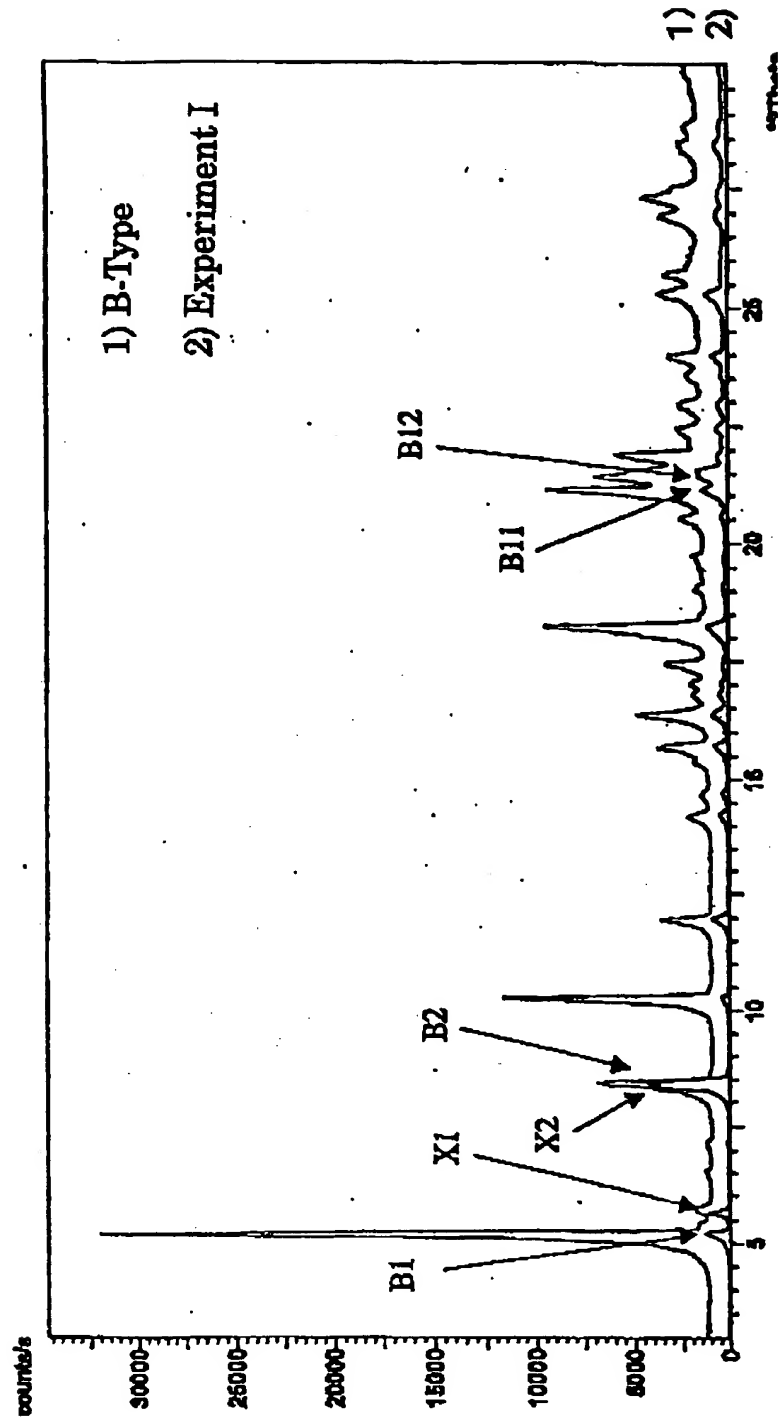


FIGURE 11

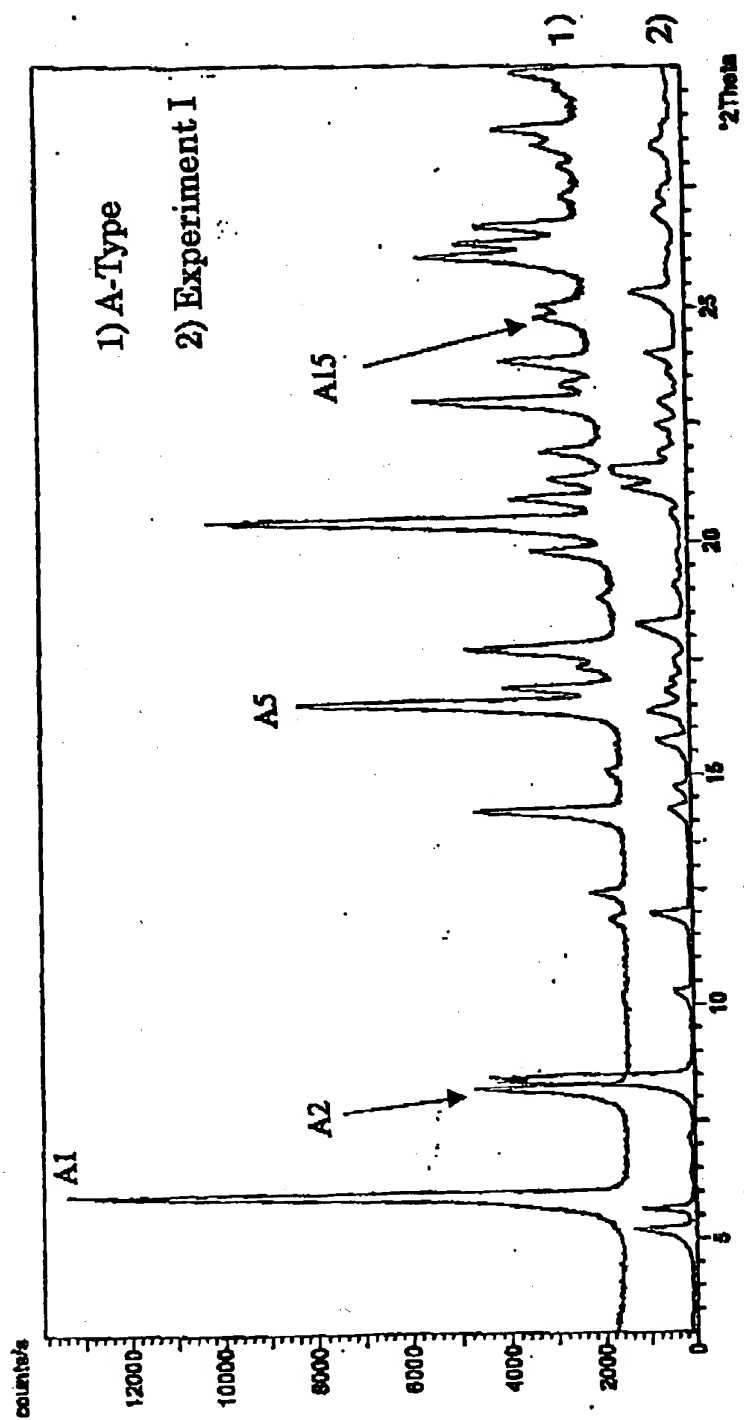




FIGURE 12

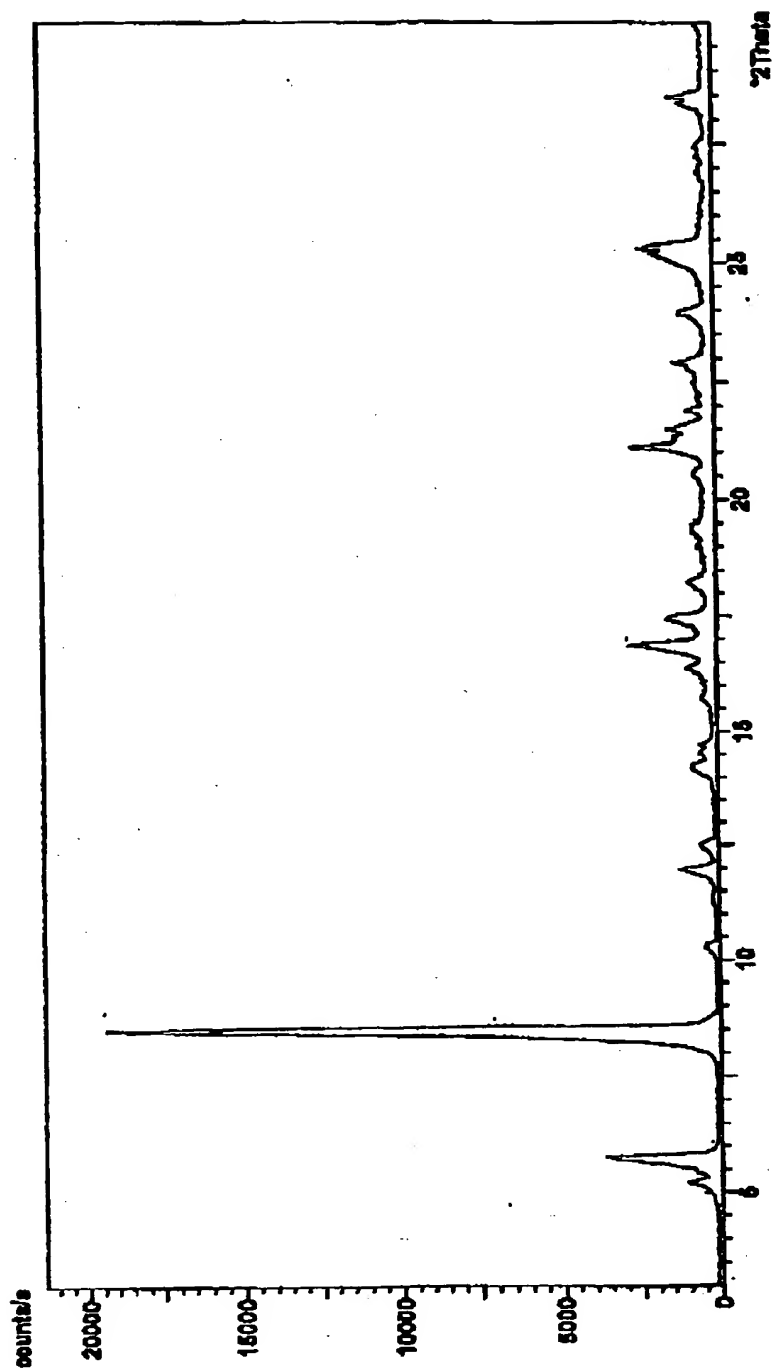


FIGURE 13

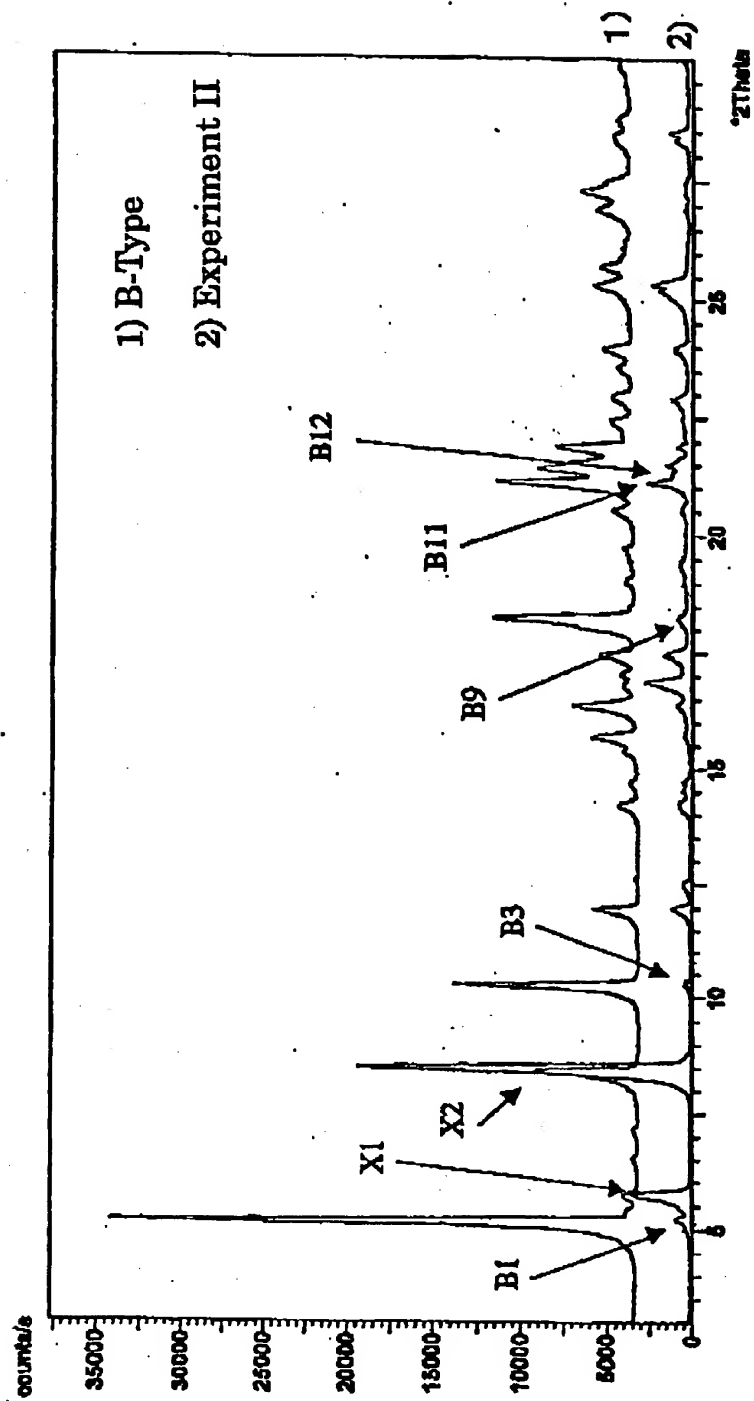
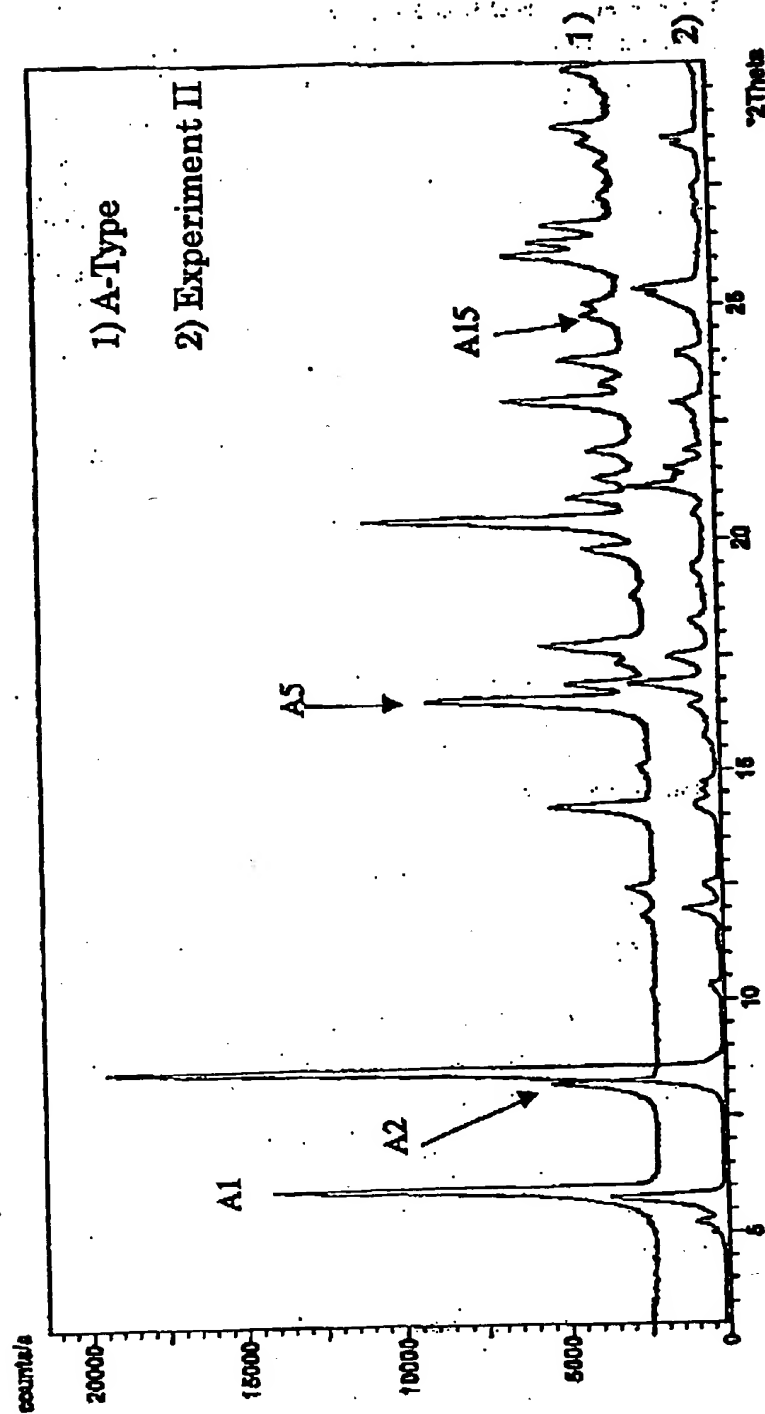
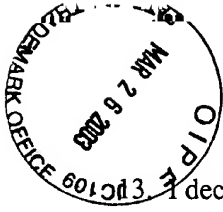


FIGURE 14





I declare further that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

14. Further Declarant saith not.

Shigeru Kawahara

Date

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(11) Publication number:

**0 279 542
A1**

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 88300742.9

(51) Int. Cl.4: C07C 101/22, C07C 99/12

(22) Date of filing: 28.01.88

(30) Priority: 19.02.87 JP 36604/87

(43) Date of publication of application:
24.08.88 Bulletin 88/34

(54) Designated Contracting States:
CH DE FR GB IT LI NL

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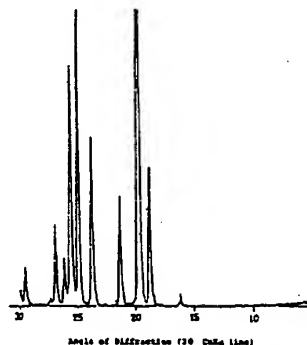
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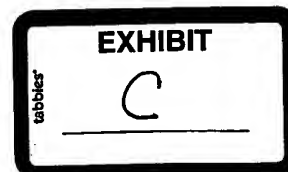
(54) Novel aspartic acid crystals and a process for the production thereof.

(57) A crystalline form of L-aspartic acid, referred to as β -L-Asp, which is obtained in the form of cylindrical crystals of low water content and which exhibit X-ray diffraction peaks at angles of diffraction (2θ , Cu K α line) of at least 18.8°, 19.7° and 25.0° when measured by a powder X-ray diffraction method, is disclosed herein.

Fig. 1



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EP 0 279 542 A1

NOVEL ASPARTIC ACID CRYSTALS AND A PROCESS FOR THE PRODUCTION THEREOF

The present invention relates to a novel crystal form of L-aspartic acid (hereinbefore referred to as L-Asp) and a process for the production thereof. L-Asp is useful as a starting material for the production of the sweetening agent Aspartame.

There is known a process for the production of L-Asp which generally comprises neutralization and crystallization of a solution obtained by an enzymatic reaction of fumaric acid.

However, L-Asp obtained by this process consists of α -type crystals having a scale or flake form. Therefore, the crystals separated by a centrifugal separator contain adsorbed water in an amount as high as 15-18% and also inevitably incorporate large amounts of impurities from the crystallization medium due to this absorbed water, and thus the purity of the obtained L-Asp is deteriorated.

Accordingly, the present inventors have intensively studied various crystallization conditions and, as a result, have come to discover a process for obtaining a novel β -type crystals of L-Asp in a cylindrical form by adding a small amount of L-phenylalanine (hereinafter referred to as L-Phe) in the presence of sodium chloride.

According to the present invention, there is provided β -type crystals of aspartic acid which exhibit X-ray diffraction peaks at angles of diffraction (2θ , Cu K α line) of at least 18.8°, 19.7° and 25.0° when measured by a powder X-ray diffraction method.

The present invention also provides a process for the production of β -type crystals of aspartic acid, which comprises crystallizing L-Asp in the co-presence of sodium chloride and phenylalanine from an aqueous solution.

Reference will be made hereinbelow to the drawings in which figures 1 and 2 are X-ray diffraction figures as measured by a powder diffraction X-ray method, figure 1 being for β -type crystals and figure 2 being for α -type crystals.

The β -type crystals contain adsorbed water in an amount of as low as 4-5% when separated by a centrifugal separator, and as a result, high purity L-Asp may be obtained with only a limited extent of incorporation of impurities. Further, drying of the obtained hydrous L-Asp is easier with the β -type crystals than with α -type crystals because the amount of adsorbed water is lower in the former case and thus it is economically more advantageous.

One method for obtaining the β -type crystals comprises adding to a solution of L-Asp sodium chloride at a concentration of from 5% by weight to the saturation point, preferably 15-25% by weight,

dissolving the sodium chloride, then adding and dissolving L-Phe and thereafter crystallizing L-Asp by cooling or neutralization.

Although the amount of L-Phe added is not particularly restricted, it can be seen, as demonstrated in Example 1, that even an amount of as small as 0.02% by weight can exert an effect. In general, the range of 0.01-0.1% by weight is suitable. The obtained β -type crystals exhibit diffraction angles (2θ , CuK α line) of at least 18.8°, 19.7° and 25.0° when measured by a powder diffraction X-ray method.

According to the present invention, as described above, by adding a small amount of L-Phe in the presence of sodium chloride during crystallization of L-Asp, industrially advantageous high purity L-Asp may be obtained.

The solutions of L-Asp may be any of those from which L-Asp has been previously obtained and crystallized, preferably an aqueous solution. The pH of the solution may be constant or variably adjusted and may preferably be from 2-8, more preferably 3-5.5.

The invention now being generally described, the same will be better understood by reference to certain specific examples which are included herein for purposes of illustration only and are not intended to be limiting of the invention or any embodiment thereof, unless specified.

Example 1

One liter of water was added to 150 g of L-Asp and 200 g of sodium chloride, and caustic soda was added thereto with stirring to adjust to pH 5.5 whereby L-Asp was dissolved. Then, 270 mg of L-Phe was added and dissolved, and thereafter 35% hydrochloric acid was added to adjust to pH 3, whereby L-Asp was crystallized by neutralization. The resultant crystals were separated by a centrifugal separator. The obtained crystals were cylindrical crystals and the adsorbed water thereof was 4.5%. Thereafter, the hydrous crystals were dried, and the powder X-ray pattern was measured to exhibit a structure of β -type crystals (see FIGURE 1).

Example 2

One liter of water was added to 100 g of L-Asp and 250 g of sodium chloride, and 35% hydrochloric acid was added thereto with stirring to dissolve L-Asp.

Thereafter, 540 mg of L-Phe was added and dissolved, and thereafter caustic soda was added to adjust the pH to 3, whereby L-Asp was crystallized by neutralization. The resultant crystals were cylindrical crystals and the adsorbed water thereof was 4.0%. Thereafter, the hydrous crystals were dried and the powder X-ray pattern was measured to exhibit a structure of β -type crystals.

7. A process according to any of claims 3 to 6, which further comprises separating said crystallized L-Asp from said aqueous solution.

Comparative Example 1

One liter of water was added to 150 g of L-Asp and 200 g of sodium chloride, and caustic soda was added thereto with stirring to adjust to pH 5.5 whereby L-Asp was dissolved. After confirming that L-Asp had been dissolved, 35% hydrochloric acid was added to adjust the pH to 3, whereby L-Asp was crystallized by neutralization. The resultant crystals were separated by a centrifugal separator. The obtained crystals were flake-formed crystals and the water adsorbed thereto was 16.5%. Thereafter, the hydrous crystals were dried, and the powder X-ray pattern was measured to exhibit a structure of α -type crystals.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.

Claims

1. β -type crystals of aspartic acid which exhibit X-ray diffraction peaks at angles of diffraction (2θ , Cu $K\alpha$ line) of at least 18.8° , 19.7° and 25.0° when measured by a powder X-ray diffraction method.
2. Crystals as claimed in claim 1, wherein the amount of adsorbed water in said crystals is at most 5% by weight.
3. A process for the production of β -type crystals of aspartic acid, which comprises crystallizing L-Asp in the co-presence of sodium chloride and phenylalanine from an aqueous solution.
4. A process according to claim 3, wherein the amount of sodium chloride ranges from a concentration of 5% by weight to the saturation point of sodium chloride in said aqueous solution.
5. A process according to claim 4, wherein the concentration of sodium chloride ranges from 15 to 25% by weight.
6. A process according to any of claims 3 to 5, wherein the amount of L-phenylalanine which is present in said aqueous solution ranges from 0.01 to 0.1% by weight.

Fig. 1

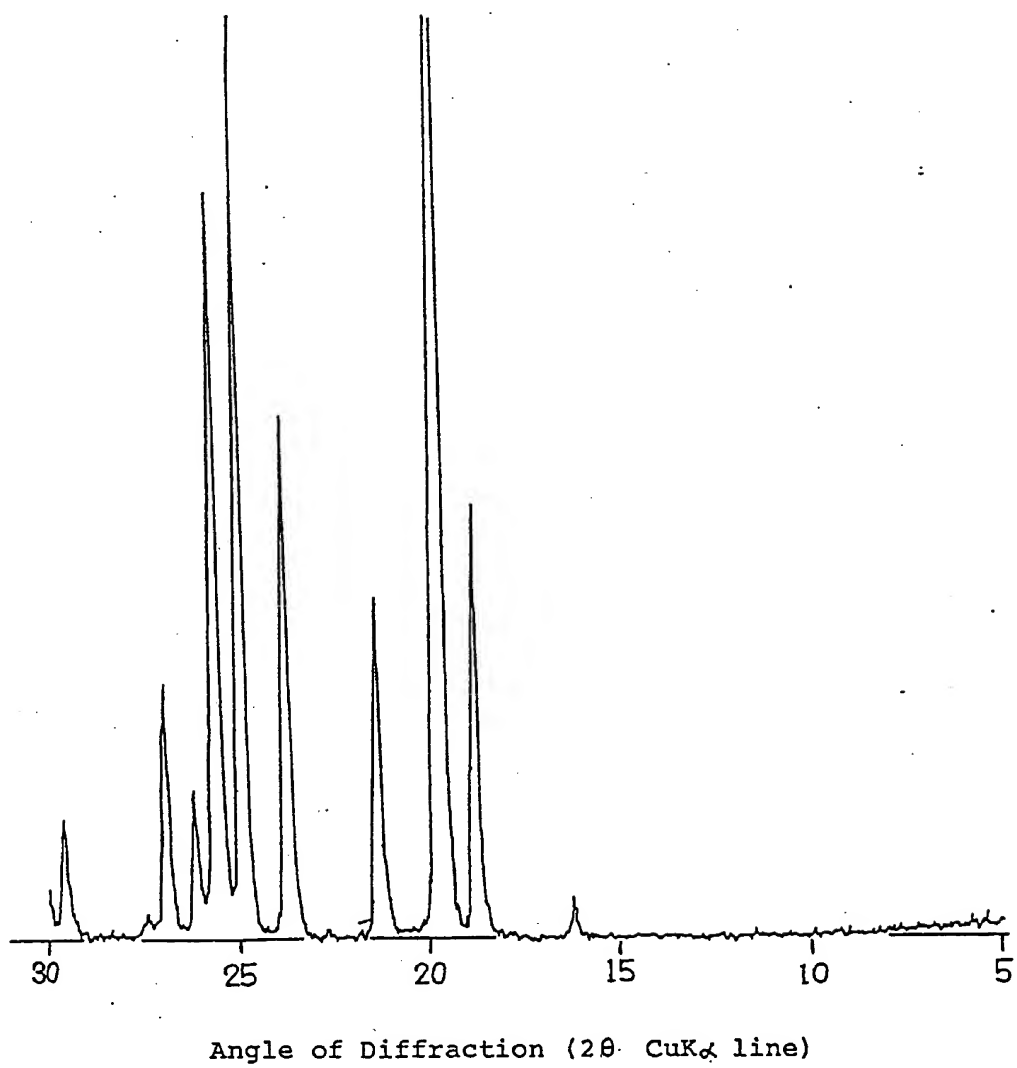
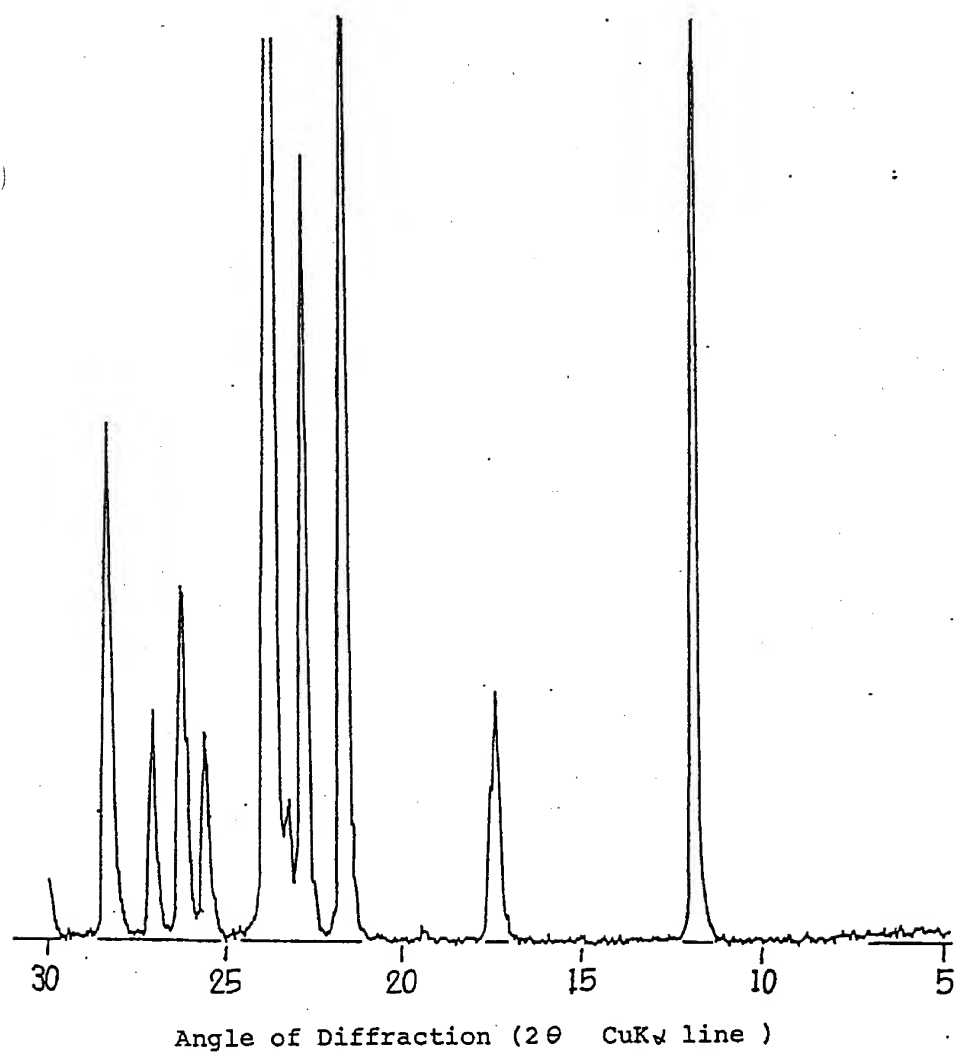


Fig. 2



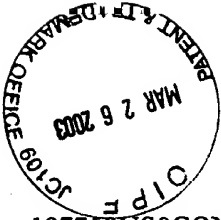


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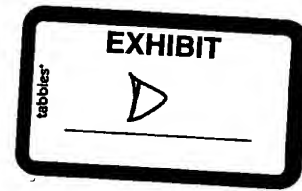
EUROPEAN SEARCH REPORT

Application number

DOCUMENTS CONSIDERED TO BE RELEVANT			EP 88300742.9
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	DE - A1 - 2 613 564 (TROPONWERKE) * Claims * --	1,7	C 07 C 101/22 C 07 C 99/12
A	US - A - 3 183 263 (J.A.FRUMP) * Totality * ----	1,3,7	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			C 07 C 101/00 C 07 C 99/00
The present search report has been drawn up for all claims			
Place of search VIENNA		Date of completion of the search 19-04-1988	Examiner HOFBAUER
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			
T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:

AKIHIRO KISHISHITA ET AL : GROUP ART UNIT: 1621

SERIAL NO: 09/708,006 :

FILED: NOVEMBER 8, 2000 : EXAMINER: ZUCKER

FOR: NOVEL ASPARTAME :
DERIVATIVE CRYSTAL AND
PROCESS FOR PRODUCING
THE SAME

DECLARATION UNDER 37 C.F.R. §1.132

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Now comes TATSUKI KASHIWAGI, who deposes and states that:

1. That I am a graduate of University of Tokyo and received my bachelor's degree in the year 1989 and Ph.D. in the year 1999.
2. That I have been employed by Ajinomoto Company Inc. for twelve years as a research scientist in the fields of the X-ray crystallography and X-ray powder diffraction ("XRPD").
3. Solid materials can be in the crystalline state or in the amorphous state. Solid materials in the crystalline state are composed of numerous fine crystals. In a single crystal, molecules or ions comprising the material are regularly and iteratively arranged. The minimum unit of the three-dimensional iteration in the crystal is called a "unit cell" and is defined as the basic parallelepiped shaped block from which the whole volume of the crystal may be built by regular assembly of these block. As shown in Figure 1 (see below), the axial lengths of the

unit cell and the angles between these axes (cell parameters) are, by convention, defined as a , b , c , α , β , and γ , respectively.

4. Even in the same material, several kinds of crystalline states (crystal forms) can exist. This phenomenon is designated as “polymorphism.” Among organic compounds, crystals which are composed of the same molecule with different amounts of crystallizing solvents like waters are often observed. The term “pseudo-polymorphism” is often used for such situation. Between the different crystal forms related by the polymorphism or pseudo-polymorphism, the molecular arrangements and interactions in the crystals are drastically different from each other.

5. As a result, polymorph or pseudo-polymorph related crystal forms possess a lot of differing characteristics, including cell parameters, crystalline morphology, solubility, melting point, density, and so on. The differences in these characteristics, especially the crystalline morphology and the solubility, largely influence the industrial processing of the crystalline materials. Therefore, even if one crystal form has already been known, the discovery of another new crystal form which has more favorable physicochemical properties should be regarded as a new invention.

6. For over a half century, XRPD has been used to identify and characterize crystalline powders. In order to discriminate between crystalline powder samples which commonly contain the same material but belong to the different crystal forms, XRPD is the most powerful and convenient method. The main reason why XRPD can be applied to the discrimination of polymorph or pseudo-polymorph crystalline powders is that each crystal form possesses its own unique cell parameters. For example, it is known that glutamic acid has two different crystal forms, that is, an α -form (Hirayama et al., Bull. Chem. Soc. Jpn., vol. 53, p. 30 (1980)) and a β -form (Hirokawa, Acta Cryst., vol. 8, p. 637 (1955)). Their cell parameters are as follows:

α -form : $a = 7.068\text{\AA}$, $b = 10.277\text{\AA}$, $c = 10.277\text{\AA}$, $\alpha = \beta = \gamma = 90^\circ$,

β -form : $a = 5.17\text{\AA}$, $b = 17.34\text{\AA}$, $c = 6.95\text{\AA}$, $\alpha = \beta = \gamma = 90^\circ$,

demonstrating that the values of a , b , and c are completely different between the two crystal forms.

7. If a crystalline sample is irradiated with a monochromatic X-ray beam, a lot of diffracted X-rays emerge at the particular angles against the incident X-ray beam (diffraction angles). The conditions for a diffracted X-ray are given by the following relation

$$n\lambda = 2d \sin \theta,$$

where, n is an integer, λ is the wavelength of the X-ray, d is the interplanar spacing between successive atomic planes in the crystal, and θ is the angle between the atomic plane and both the incident and diffracted beam. This fundamental relation is known as the Bragg equation or Bragg's law and is schematized in Figure 2 (see below). As shown in Figure 2, the diffraction angle can be considered as 2θ .

8. In the measurement of XRPD, the wavelength of the X-ray is usually fixed to a particular value, most commonly, to 1.5418\AA (the characteristic X-ray of Cu K α). Therefore, the diffraction angle 2θ can be regarded as a function of d . The interplanar spacing d is represented by the following relation:

$$1/d = (h^2a^{*2} + k^2b^{*2} + l^2c^{*2} + 2klb^*c^* \cos \alpha^* + 2lhc^*a^* \cos \beta^* + 2hka^*b^* \cos \gamma^*)^{1/2},$$

where, h , k , and l are integers, and a^* , b^* , c^* , α^* , β^* , and γ^* are the parameters which are closely related to the cell parameters by the following equations

$$a^* = bc \sin \alpha / V$$

$$b^* = ca \sin \beta / V$$

$$c^* = ab \sin \gamma / V$$

$$\cos \alpha^* = (\cos \beta \cos \gamma - \cos \alpha) / \sin \beta \sin \gamma$$

$$\cos \beta^* = (\cos \gamma \cos \alpha - \cos \beta) / \sin \gamma \sin \alpha$$

$$\cos \gamma^* = (\cos \alpha \cos \beta - \cos \gamma) / \sin \alpha \sin \beta,$$

where, V is the volume of the unit cell. Thus, the diffracted X-rays of a crystalline powder occurs only at the particular 2θ angles which are determined by its cell parameters.

Resultantly, the XRPD patterns of the same materials belonging to different crystal forms demonstrate diffraction peaks with discordant 2θ angles.

9. Of course, the intensities of the diffracted X-rays are also important for identifying and characterizing crystalline powders. In the case when the effects of the apparatus, the peak overlap, and the crystalline morphology etc. are negligible, the intensities of the diffraction peaks basically depend on the molecular arrangement in the crystal, suggesting that the strength order of the diffraction peaks is basically conserved in the XRPD patterns of one crystal form.

10. It is a conventional identification method for a specific crystal to find crystal(s) having the same three strongest peaks of the specific crystal on XRPD data bases and, when found, to analyze coincidence of other peaks between them. In general cases, the crystal can be identified when the strongest eight peaks are coincident. In the case of the specific crystals of a particular compound, for instance, A-type crystals, C-type crystals, or other types crystals of neotame, it is possible to identify one of them by using one strong and characteristic peak.

11. Figure 3 (see below) demonstrates the XRPD patterns of *N*-(3,3-dimethylbutyl)-APM (Neotame). In the XRPD of the A-type crystal form which is obtained using the processes and recrystallization methods described in U.S. Patent No. 5,480,668 (Nofre et al), the intense diffraction peaks occur at the 2θ angles of 6.0° , 8.2° , 14.2° , 16.5° , 16.8° , 17.7° , 20.3° , 20.8° , 22.8° , 26.0° , 26.2° , and 26.6° . On the other hand, the intense diffraction peaks of the XRPD of the C-type crystal form obtained by the method disclosed in U.S. Patent Application Serial No. 09/708,006 occur at the 2θ angles of 6.3° , 7.1° , 15.1° , 17.3° , 17.7° ,

19.8°, 20.3°, 21.7°, 22.7°, and 26.5°. Based on the 2θ angles of the diffractions, the two XRPD patterns are clearly different from each other. The XRPD patterns of D-type and E-type crystals are also completely different from that of C-type crystals. These XRPD patterns were collected using the same apparatus (Philips, X'Pert-MPD), suggesting that the systematic error of 2θ angles is negligible and that the discordance of 2θ angles is derived from the differences of the crystals themselves. Crystals of Neotame can be identified as C-type crystals of neotame when a diffraction peak at 7.1° is observed, because this peak is characteristic of the C-type crystal form. The differences in the other diffraction peaks observed between the two types of crystals further support the conclusion that the C-type crystal form is a completely different crystal form from the A-type crystal form.

12. Furthermore, the A-type and C-type crystals contain 3-6 wt% and <3 wt% of water, respectively. These results suggest that these Neotame crystals are related by the pseudo-polymorphism relationship. However, on the other hand, the solubility of the C-type crystal is larger than that of the A-type crystal.

13. In conclusion, from the above observations, I believe that the Neotame C-type crystal form is a novel material with more favorable properties and it should be regarded as the new invention.

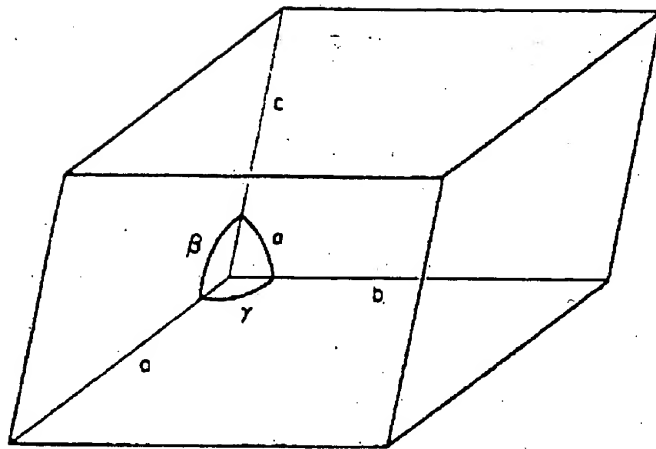


Figure 1. Unit Cell of a crystal

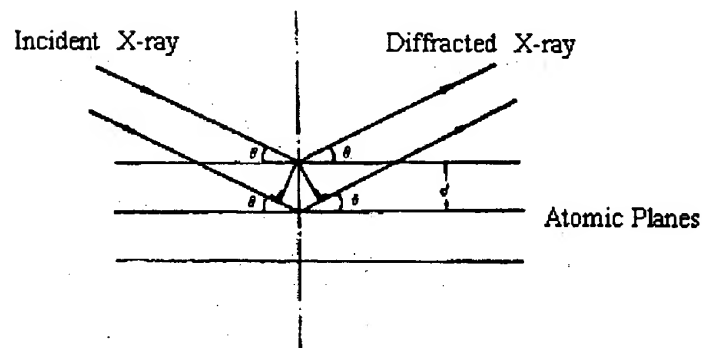


Figure 2. Bragg Equation

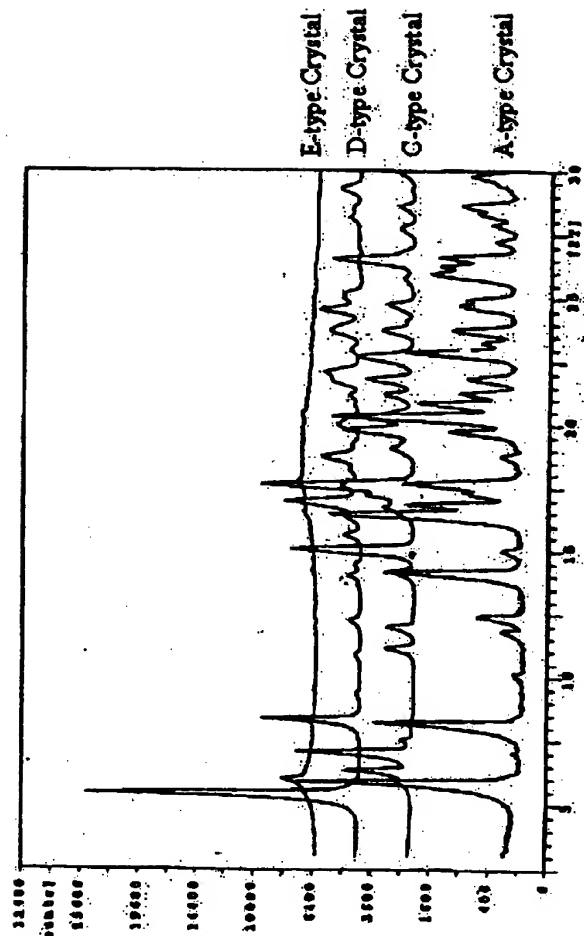


Figure 3. XRPD Patterns of Neotame Crystals

14. I declare further that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

15. Further Declarant saith not.

Tatsuki Kashiwagi

TATSUKI KASHIWAGI

Date October 31, 2002